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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 15/12, C07K 13/00, C12N 5/10, G01N 33/68

A1

(11) International Publication Number: WO 94/13799

(43) International Publication Date: 23 June 1994 (23.06.94)

(21) International Application Number: PCT/GB93/02506

(22) International Filing Date: 7 December 1993 (07.12.93)

(30) Priority Data:

9225776.5	10 December 1992 (10.12.92)	GB
9318528.8	7 September 1993 (07.09.93)	GB

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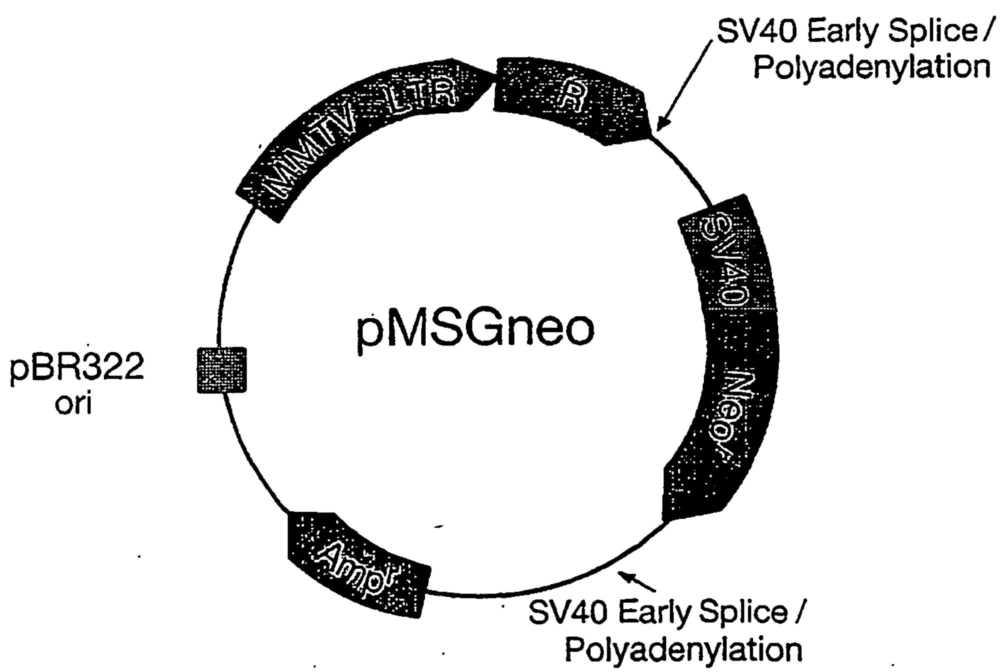
(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: STABLY TRANSFECTED CELL LINES EXPRESSING GABA-A RECEPTORS



(57) Abstract

The present invention relates to a stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor, which receptor comprises the $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ subunit combination; to membrane preparations derived from cultures thereof; and to the use of the cell line in designing and developing $GABA_A$ receptor subtype-selective medicaments.

STABLY TRANSFECTED CELL LINES EXPRESSING GABA-A RECEPTORS

This invention concerns a cell line, and in particular relates to a stable cell line capable of expressing human or animal $GABA_A$ receptors. The invention further concerns the cloning of novel cDNA sequences encoding particular subunits of the human $GABA_A$ receptor. In addition, the invention relates to the use of the cell line in a screening technique for the design and development of subtype-specific medicaments.

Gamma-amino butyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. It mediates fast synaptic inhibition by opening the chloride channel intrinsic to the $GABA_A$ receptor. This receptor comprises a multimeric protein of molecular size 230-270 kDa with specific binding sites for a variety of drugs including benzodiazepines, barbiturates and β-carbolines, in addition to sites for the agonist ligand GABA (for reviews see Stephenson, Biochem. J., 1988, 249, 21; Olsen and Tobin, Faseb J., 1990, 4, 1469; and Sieghart, Trends in Pharmacol. Sci., 1989, 10, 407).

Molecular biological studies demonstrate that the receptor is composed of several distinct types of subunit, which are divided into four classes (α, β, γ, and δ) based on their sequence similarities. To date, six types of α (Schofield et al., Nature (London), 1987, 328, 221; Levitan et al., Nature (London), 1988, 335, 76; Ymer et al., EMBO J., 1989, 8, 1665; Pritchett & Seeberg, J. Neurochem., 1990, 54, 802; Luddens et al., Nature (London), 1990, 346, 648; and Khrestchatisky et al., Neuron, 1989, 3, 745), three types of β (Ymer et al., EMBO J., 1989, 8, 1665), two types of γ (Ymer et al., EMBO J., 1990, 9, 3261; and Shivers et al., Neuron, 1989, 3, 327) and one δ subunit (Shivers et al., Neuron, 1989, 3, 327) have been identified.

The differential distribution of many of the subunits has been characterised by in situ hybridisation (Sequier et al., Proc. Natl. Acad. Sci. USA, 1988, 85, 7815; Malherbe et al., J. Neurosci., 1990, 10, 2330; and Shivers et al., Neuron, 1989, 3, 327) and this has permitted it to be speculated which subunits, by their co-localisation, could theoretically exist in the same receptor complex.

Various combinations of subunits have been co-transfected into cells to identify synthetic combinations of subunits whose pharmacology parallels that of bona fide $GABA_A$ receptors in vivo (Pritchett et al., Science, 1989, 245, 1389; Malherbe et al., J. Neurosci., 1990, 10, 2330; Pritchett and Seeberg, J. Neurochem., 1990, 54, 1802; and Luddens et al., Nature (London), 1990, 346, 648). This approach has revealed that, in addition to an α and β subunit, either γ_1 or γ_2 (Pritchett et al., Nature (London), 1989, 338, 582; Ymer et al., EMBO J., 1990, 9, 3261; and Malherbe et al., J. Neurosci., 1990, 10, 2330) or γ_3 (Herb et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 1433; Knoflach et al., FEBS Lett., 1991, 293, 191; and Wilson-Shaw et al., FEBS Lett., 1991, 284, 211) is also generally required to confer benzodiazepine sensitivity, and that the benzodiazepine pharmacology of the expressed receptor is largely dependent on the identity of the α and γ subunits present. Receptors containing a δ subunit (i.e. $\alpha\beta\delta$) do not appear to bind benzodiazepines (Shivers et al., Neuron, 1989, 3, 327). Combinations of subunits have been identified which exhibit the pharmacological profile of a BZ_1 type receptor ($\alpha_1\beta_1\gamma_2$) and a BZ_2 type receptor ($\alpha_2\beta_1\gamma_2$ or $\alpha_3\beta_1\gamma_2$, Pritchett et al., Nature (London), 1989, 338, 582), as well as two $GABA_A$ receptors with a novel pharmacology, $\alpha_5\beta_2\gamma_2$ (Pritchett and Seeberg, J. Neurochem., 1990, 54, 1802) and $\alpha_6\beta_2\gamma_2$ (Luddens et al., Nature (London), 1990, 346, 648). Although the pharmacology of these expressed receptors appears similar

to that of those identified in brain tissue by radioligand binding, it has nonetheless not been shown that these receptor subunit combinations exist in vivo.

The present invention is concerned with the production of permanently transfected cells containing the $GABA_A$ receptor, which will be useful for screening for drugs which act on this receptor. The $GABA_A$ receptor has previously been expressed in Xenopus oocytes (Sigel et al., Neuron, 1990, 5, 703-711) and in transiently transfected mammalian cells (Pritchett et al., Science, 1989, 245, 1389-1392). However, both of those systems involve transient expression and are unsuitable for screening purposes.

We have now achieved the stable expression of the receptor.

Accordingly, the present invention provides a stably co-transfected eukaryotic cell line capable of expressing a $GABA_A$ receptor, which receptor comprises at least one alpha, one beta and one gamma subunit.

This has been achieved by co-transfecting cells with three expression vectors, each harbouring cDNAs encoding for an α, β or γ $GABA_A$ receptor subunit. In a further aspect, therefore, the present invention provides a process for the preparation of a eukaryotic cell line capable of expressing a $GABA_A$ receptor, which comprises stably co-transfecting a eukaryotic host cell with at least three expression vectors, one such vector harbouring the cDNA sequence encoding for an alpha, another such vector harbouring the cDNA sequence encoding for a beta, and a third such vector harbouring the cDNA sequence encoding for a gamma $GABA_A$ receptor subunit. The stable cell-line which is established expresses an $\alpha\beta\gamma$ $GABA_A$ receptor. Each receptor thereby expressed, comprising a unique combination of α, β and γ subunits, will be referred to hereinafter as a $GABA_A$ receptor "subunit combination". Pharmacological and electrophysiological data confirm that the recombinant

$\alpha\beta\gamma$ receptor expressed by the cells of the present invention has the properties expected of a native $GABA_A$ receptor.

Expression of the $GABA_A$ receptor may be accomplished by a variety of different promoter-expression systems in a variety of different host cells. The eukaryotic host cells suitably include yeast, insect and mammalian cells. Preferably the eukaryotic cells which can provide the host for the expression of the receptor are mammalian cells. Suitable host cells include rodent fibroblast lines, for example mouse Ltk^-, Chinese hamster ovary (CHO) and baby hamster kidney (BHK); HeLa; and HEK293 cells. It is necessary to incorporate at least one α, one β and one γ subunit into the cell line in order to produce the required receptor. Within this limitation, the choice of receptor subunit combination is made according to the type of activity or selectivity which is being screened for. For example, benzodiazepines (designated BZ) represent one class of drugs which act upon the $GABA_A$ receptor. The presence of an α_1 subunit is specific for a class of benzodiazepines having the pharmacology designated BZ_1; whereas α_2 to α_5 define different pharmacological profiles, broadly designated as BZ_2. The type of β subunit is not critical in defining the class of benzodiazepine, although a β subunit is required. The γ subunit is also important in defining BZ selectivity. It is likely that differentiation between α subunit selectivity is conferred by the identity of the particular γ subunit present.

In order to employ this invention most effectively for screening purposes, it is preferable to build up a library of cell lines, each with a different combination of subunits. Typically a library of 5 or 6 cell line types is convenient for this purpose. Preferred subunit combinations include: $\alpha_1\beta_1\gamma_2$; $\alpha_1\beta_2\gamma_2$;

$\alpha_2\beta_1\gamma_1$; $\alpha_2\beta_1\gamma_2$; $\alpha_2\beta_1\gamma_3$; $\alpha_3\beta_1\gamma_2$; $\alpha_3\beta_1\gamma_3$; $\alpha_4\beta_1\gamma_2$; $\alpha_5\beta_1\gamma_2$; and $\alpha_6\beta_1\gamma_2$; especially $\alpha_1\beta_1\gamma_{2L}$.

In a particular embodiment, the present invention provides a stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_1\beta_3\gamma_2$ subunit combination.

In a further embodiment, the present invention provides a stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_2\beta_3\gamma_2$ subunit combination.

In a still further embodiment, the present invention provides a stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_5\beta_3\gamma_2$ subunit combination.

In yet further embodiments, the present invention provides stably co-transfected eukaryotic cell lines capable of expressing human $GABA_A$ receptors comprising the $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ subunit combinations.

The DNAs for the receptor subunits can be obtained from known sources, and are generally obtained as specific nucleotide sequences harboured by a standard cloning vector such as those described, for example, by Maniatis et al. in Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, New York, 2nd edition, 1989. Preferably the cDNA sequences are derived from the human gene. However, for screening purposes, cDNAs from other species are also suitable, such as bovine or rat DNA. Known sources of $GABA_A$ receptor subunit cDNAs are as follows:

α_1 bovine	)	Schofield et al., Nature, 1987, 328,
β_1 bovine	)	221-227.
α_1 human	)	Schofield et al., FEBS Lett., 1989, 244,
β_1 human	)	361-364.

α_2 rat	Khrestchatisky et al., J. Neurochem., 1991, 56, 1717.
α_2 bovine)	Levitan et al., Nature, 1988, 335,
α_3 bovine)	76-79.
α_4 rat	Wisden et al., FEBS Lett., 1991, 289, 227.
α_4 bovine	Ymer et al., FEBS Lett., 1989, 258, 119-122.
α_5 rat	Pritchett and Seeburg, J. Neurochem., 1990, 54, 1802-1804.
α_6 rat)	Luddens et al., Nature, 1990, 346,
α_6 bovine)	648-651.
β_2 bovine)	Ymer et al., EMBO J., 1989, 8, 1665-1670.
β_2 rat)	
β_3 bovine)	
β_3 rat)	
β_3 human	Wagstaff et al., Am. J. Hum. Genet., 1991, 49, 330.
γ_1 human)	Ymer et al., EMBO J., 1990, 9, 3261-3267.
γ_1 rat)	
γ_1 bovine)	
γ_2 human	Pritchett et al., Nature, 1989, 338, 582-585.
γ_2 bovine	Whiting et al., Proc. Natl. Acad. Sci. USA, 1990, 57, 9966-9970.
γ_3 rat	Herb et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 1433; and

	Knoflach et al., FEBS Lett., 1991, 293, 191.
γ_3 mouse	Wilson-Shaw et al., FEBS Lett., 1991, 284, 211.
δ rat	Shivers et al., Neuron, 1989, 3, 327.

Certain cDNA sequences encoding various subunits of the human $GABA_A$ receptor have hitherto been unavailable. These include in particular the sequences encoding the α_2, α_3, α_5, α_6 and β_2 subunits, which nucleotide sequences are accordingly novel. We have now ascertained the cDNA sequences of the α_2, α_3, α_5, α_6 and β_2 subunits of the human $GABA_A$ receptor. These nucleotide sequences, together with the deduced amino acid sequences corresponding thereto, are depicted in Figures 2 to 6 of the accompanying drawings. The present invention accordingly provides in several additional aspects DNA molecules encoding the α_2, α_3, α_5, α_6 and β_2 subunits of the human $GABA_A$ receptor comprising all or a portion of the sequences depicted in Figures 2, 3, 4, 5 and 6 respectively, or substantially similar sequences.

The sequencing of the novel cDNA molecules in accordance with the invention can conveniently be carried out by the standard procedure described in accompanying Example 3; or may be accomplished by alternative molecular cloning techniques which are well known in the art, such as those described by Maniatis et al. in Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, New York, 2nd edition, 1989.

In another aspect, the invention provides a recombinant expression vector comprising the nucleotide sequence of a $GABA_A$ receptor subunit together with additional sequences capable of directing the synthesis of the said $GABA_A$ receptor subunit in cultures of stably co-transfected eukaryotic cells.

The term "expression vectors" as used herein refers to DNA sequences that are required for the transcription of cloned copies of recombinant DNA sequences or genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, blue-green algae, yeast cells, insect cells, plant cells and animal cells. Specifically designed vectors allow the shuttling of DNA between bacteria-yeast, bacteria-plant or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selective markers, a limited number of useful restriction enzyme sites, a high copy number, and strong promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and to initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

The term "cloning vector" as used herein refers to a DNA molecule, usually a small plasmid or bacteriophage DNA capable of self-replication in a host organism, and used to introduce a fragment of foreign DNA into a host cell. The foreign DNA combined with the vector DNA constitutes a recombinant DNA molecule which is derived from recombinant technology. Cloning vectors may include plasmids, bacteriophages, viruses and cosmids.

The recombinant expression vector in accordance with the invention may be prepared by inserting the nucleotide sequence of the chosen $GABA_A$ subunit into a suitable precursor expression vector (hereinafter referred to as the "precursor vector") using conventional recombinant DNA methodology known from the art. The precursor vector may be obtained commercially, or

constructed by standard techniques from known expression vectors. The precursor vector suitably contains a selection marker, typically an antibiotic resistance gene, such as the neomycin or ampicillin resistance gene. The precursor vector preferably contains a neomycin resistance gene, adjacent the SV40 early splicing and polyadenylation region; an ampicillin resistance gene; and an origin of replication, e.g. pBR322 ori. The vector also preferably contains an inducible promoter, such as MMTV-LTR (inducible with dexamethasone) or metallothionin (inducible with zinc), so that transcription can be controlled in the cell line of this invention. This reduces or avoids any problem of toxicity in the cells because of the chloride channel intrinsic to the $GABA_A$ receptor.

One suitable precursor vector is pMAMneo, available from Clontech Laboratories Inc. (Lee et al., Nature, 1981, 294, 228; and Sardet et al., Cell, 1989, 56, 271). Alternatively the precursor vector pMSGneo can be constructed from the vectors pMSG and pSV2neo as described in Example 1 herein.

The recombinant expression vector of the present invention is then produced by cloning the $GABA_A$ receptor subunit cDNA into the above precursor vector. The required receptor subunit cDNA is subcloned from the vector in which it is harboured, and ligated into a restriction enzyme site, e.g. the HindIII site, in the polylinker of the precursor vector, for example pMAMneo or pMSGneo, by standard cloning methodology known from the art, and in particular by techniques analogous to those described in Example 1, step (b) herein. Before this subcloning, it is often advantageous, in order to improve expression, to modify the end of a subunit cDNA with additional 5' untranslated sequences, for example by modifying the 5' end of the γ_{2L} subunit DNA by addition of 5' untranslated region sequences from the α_1 subunit DNA.

One suitable expression vector of the present invention is illustrated in Fig. 1 of the accompanying drawings, in which R represents the nucleotide sequence of a given alpha, beta or gamma subunit of the $GABA_A$ receptor, and the remainder of the expression vector depicted therein is derived from the precursor vector pMSGneo and constructed as described in accompanying Example 1, steps (a) and (b).

For each cell line of the present invention, three such vectors will be necessary, one containing an α subunit, one containing a β subunit, and the third containing a γ subunit.

Cells are then co-transfected with the desired combination of three expression vectors. There are several commonly used techniques for transfection of eukaryotic cells <u>in vitro</u>. Calcium phosphate precipitation of DNA is most commonly used (Bachetti <u>et al</u>., <u>Proc. Natl. Acad. Sci. USA</u>, 1977, <u>74</u>, 1590-1594; Maitland <u>et al</u>., <u>Cell</u>, 1977, <u>14</u>, 133-141), and represents a favoured technique in the context of the present invention.

A small percentage of the host cells takes up the recombinant DNA. In a small percentage of those, the DNA will integrate into the host cell chromosome. Because the neomycin resistance gene will have been incorporated into these host cells, they can be selected by isolating the individual clones which will grow in the presence of neomycin. Each such clone is then tested to identify those which will produce the receptor. This is achieved by inducing the production, for example with dexamethasone, and then detecting the presence of receptor by means of radioligand binding.

In a further aspect, the present invention provides protein preparations of $GABA_A$ receptor subunit combinations, especially human $GABA_A$ receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells. The invention also provides

preparations of membranes containing subunit combinations of the $GABA_A$ receptor, especially human $GABA_A$ receptor subunit combinations, derived from cultures of stably transfected eukaryotic cells. In particular, the protein preparations and membrane preparations according to the invention will suitably contain the $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_2$, $\alpha_3\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ subunit combinations of the human $GABA_A$ receptor, and will preferably contain a human $GABA_A$ receptor consisting of the $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_3\gamma_{2S}$, $\alpha_5\beta_3\gamma_{2S}$, $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_3\beta_3\gamma_{2S}$ or $\alpha_6\beta_3\gamma_{2S}$ subunit combinations. In an especially preferred embodiment, the invention provides cell membranes containing a human $GABA_A$ receptor consisting of the $\alpha_1\beta_1\gamma_{2L}$, $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_3\gamma_{2S}$, $\alpha_5\beta_3\gamma_{2S}$, $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_3\beta_3\gamma_{2S}$ or $\alpha_6\beta_3\gamma_{2S}$ subunit combinations isolated from stably transfected mouse Ltk^- fibroblast cells.

The cell line, and the membrane preparations therefrom, according to the present invention have utility in screening and design of drugs which act upon the $GABA_A$ receptor, for example benzodiazepines, barbiturates, β-carbolines and neurosteroids. The present invention accordingly provides the use of the cell line described above, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon the $GABA_A$ receptor. Of particular interest in this context are molecules capable of interacting selectively with $GABA_A$ receptors made up of varying subunit combinations. As will be readily apparent, the cell line in accordance with the present invention, and the membrane preparations derived therefrom, provide ideal systems for the study of structure, pharmacology and function of the various $GABA_A$ receptor subtypes.

The following non-limiting Examples illustrate the present invention.

EXAMPLE 1

PREPARATION OF $\alpha_1\beta_1\gamma_{2L}$ TRANSFECTED CELLS

a) Construction of eukaryotic expression vector pMSGneo

The approx. 2500 base pair HindIII-EcoRI fragment of the vector pMSG (purchased from Pharmacia Biosystems Limited, Milton Keynes, United Kingdom), containing the gpt structural gene and SV40 polyadenylation signals was replaced by the approx. 2800 base pair HindIII-EcoRI fragment of pSV2neo (Southern, P.J. and Berg, P.J., Molecular and Applied Genetics, 1, 327-341, 1982) containing the neomycin resistance gene Neo^r and SV40 polyadenylation signals. The EcoRI and HindIII sites were then removed by restriction digesting, blunt ending with klenow polymerase, and religating. EcoRI and HindIII cloning sites were then inserted at the XhoI and SmaI sites of the polylinker by conventional techniques using EcoRI and HindIII linkers.

b) Cloning of subunit cDNAs into pMSGneo

Bovine α_1 and β_1 $GABA_A$ receptor cDNAs were obtained from the Molecular Neurobiology Unit, MRC Centre, Hills Road, Cambridge (Scholfield, P. et al. Nature, 328, 221-227, 1987). Bovine γ_2 cDNA was cloned by the method of Whiting, P. et al. (Proc. Natl. Acad. Sci. USA, 87, 9966-9970, 1990). Bovine α_1 was subcloned from pbGRαsense by digestion with EcoRI, blunt ending the DNA with klenow polymerase, addition of HindIII linkers by ligation, digestion with HindIII and ligation into the HindIII site of pMSGneo. Bovine β_1 was subcloned from pbGRβsense by restriction digestion with EcoRI (partial digestion), klenow polymerase blunt ending, ligation of HindIII linkers, restriction digestion with HindIII and ligation into HindIII site of pMSGneo. Before subcloning into pMSGneo, the bovine γ_2 cDNA was modified from the

published sequence as follows. The 5' untranslated region of the bovine α_1 cDNA (bases 60-200 of the published sequence) was added to the 5' end of the published γ_2 sequence by amplifying the α_1 untranslated region using polymerase chain reaction, and then subcloning the product into the 5' BamHI (site in the polylinker of the Bluescript Sk^- cloning vector; Bluescript vector purchased from Stratagene, San Diego, U.S.A.) HindIII sites of the γ_2 cDNA. The modified γ_2 cDNA was then subcloned into pMSGneo by digestion with XbaI (site in the polylinker of the cloning vector), blunt ending with klenow polymerase, ligation of XhoI linkers, digestion with XhoI (site in the polylinker of the cloning vector), and ligation into XhoI site of pMSGneo.

c) <u>Co-transfection of mouse Ltk^- cells</u>

Ltk^- cells were obtained from the Salk Institute for Biological Studies, San Diego, California. Cells were grown at 37°C, 5-8% CO_2, in Modified Eagles Medium containing penicillin, streptomycin and 10% fetal calf serum. The expression vector harbouring the $GABA_A$ receptor subunit DNAs for co-transfection was prepared by a standard protocol (Chen, C. and Okayama, H., <u>BioTechniques</u>, <u>6</u>, 632-638, 1988). For co-transfection, Ltk^- cells were plated in dishes (approx. $2x10^5$ cells/dish) and grown overnight. The transfection was performed by calcium phosphate precipitation using a kit (purchased from 5 Prime -> 3 Prime Products, Westchester, Pennsylvania). Co-transfection was performed according to manufacturers' instructions, using 5µg of each subunit DNA construct per 10cm dish of cells. After 2 days in culture the cells were divided 1:8 into culture medium containing 1mg/ml neomycin [Geneticin (obtainable from Gibco BRL, Paisley, Scotland, U.K.)]. After a further week the concentration was increased to 1.5mg/ml, and then 2mg/ml 1 week after that. Resistant clones of cells

were isolated and subcloned using cloning cylinders. Subclones were analysed using radioligand binding: subclones were grown in 10cm culture dishes, and when confluent changed into culture medium containing 1μM dexamethasone (obtainable from Sigma Chemical Company, Poole, Dorset, United Kingdom). 3-5 days later the cells were harvested, membranes prepared and used for radioligand binding (see Example 2, step (a) below) using the benzodiazepine antagonist ^{3}H Ro15-1788 (obtained from New England Nuclear, Du Pont (U.K.) Ltd, Stevenage, United Kingdom). The clone expressing the highest amount of ^{3}H Ro15-1788 binding was subcloned from a single cell by limiting dilution. The resultant clonal population of cells described below is referred to as population A.

EXAMPLE 2

CHARACTERIZATION OF $\alpha_1\beta_1\gamma_{2L}$ TRANSFECTED CELLS

a) Radioligand binding

The nature of the recombinant $\alpha_1\beta_1\gamma_{2L}$ $GABA_A$ receptors prepared as described in Example 1 was addressed by characterization of the benzodiazepine (BZ) binding pharmacology, using the BZ antagonist ^{3}H Ro15-1788. For radioligand binding assays, cells which had been induced by culture in dexamethasone containing medium for 3-5 days were scraped off into 50mM Tris, pH7.5, 100mM NaCl in the form of Tris buffered saline (TBS) and pelleted (20,000rpm, Sorvall RC5C centrifuge). The cell pellet was resuspended in 50mM Tris, pH7.5, homogenised using an Ultra-Turrax homogeniser and then pelleted as above. This was repeated once more, and the cells then resuspended in TBS (0.4ml per original 10cm dish of cells). Radioligand binding was performed in 0.1ml final volume TBS, containing 5-15 fmols of ^{3}H Ro15-

1788 binding sites. After 1 hour incubation on ice the membranes were harvested onto filters using a Brandel cell harvester, washed with cold TBS, and bound radioactivity determined by scintillation counting. The recombinant $\alpha_1\beta_1\gamma_{2L}$ receptors bound ^{3}H Ro15-1788 with high affinity (K_D 0.4nM), at levels of up to 200fmols/10cm dish of cells. No binding was seen to either untransfected Ltk$^-$ cells, or population A cells which had not been induced by addition of dexamethasone to the culture medium, confirming that the ^{3}H Ro15-1788 was binding to recombinant $\alpha_1\beta_1\gamma_2$ $GABA_A$ receptors. The ^{3}H Ro15-1788 binding was inhibited by flunitrazepam, CL218872, FG8205, βCCM, zolpidem and Ro15-4513, confirming the BZ pharmacology of the recombinant receptor. Since it is established that only $GABA_A$ receptors containing an α, a β and a γ subunit exhibit BZ binding (Pritchett, D. <u>et al</u>., <u>Nature</u>, <u>338</u>, 582-585, 1989) these data confirm the nature of the recombinant $\alpha_1\beta_1\gamma_2$ $GABA_A$ receptors expressed by population A cells.

b) <u>Electrophysiology</u>

The nature of the $GABA_A$ receptor expressed by population A cells has been extensively characterised by electrophysiological techniques, using whole cell patch clamp. Only cells induced by culture in the presence of dexamethasone showed responses to GABA. Concentration response curves to GABA gave a log EC_{50} of 5.2, and a Hill coefficient of 1.9. The response to GABA was potentiated by BZs flunitrazepam and CL218872, by the barbiturate pentobarbitone, and by the steroid alphaxalone. The response to GABA was antagonised by both bicuculline and picrotoxin. All these electrophysiological data confirm that the recombinant $GABA_A$ receptor expressed by population A cells has all of the properties expected of a <u>bona fide</u> $GABA_A$ receptor.

EXAMPLE 3

ISOLATION AND SEQUENCING OF cDNAS ENCODING HUMAN $GABA_A$ RECEPTOR α_2, α_3, α_5, α_6 & β_2 SUBUNITS

a) cDNA libraries

cDNAs were cloned from human foetal brain (α_2, α_3), hippocampal (α_5, β_2) and cerebellum (α_6) lambda bacteriophage cDNA libraries. All cDNA libraries were constructed in the lambdaZAP vector, and were purchased from Stratagene (San Diego, California). For screening, the cDNA libraries were plated according to the manufacturer's instructions, at 40,000 pfu per 137 mm plate. Filter lifts were taken using Hybond N filters (Amersham) according to the manufacturer's instructions.

b) Isolation of cDNA encoding human α_2 subunit

A bovine α_2 cDNA (obtained from E. Barnard, Molecular Neurobiology, University of Cambridge, Hills Road, Cambridge; Levitan et al., Nature, 1988, 335, 76) was labelled to high specific activity (>1.10^9 cpm/μg) with ^{32}P by random priming and used as a probe. Library filters (8 replica filters) were prehybridised for 3-6 hours at 42°C in 5x SSPE (1x SSPE is 0.18M NaCl, 0.01M Na_3PO_4 [pH7.4], 1mM EDTA), 5x Denhardt's solution, 100 μg/ml salmon sperm DNA, 0.1% sodium dodecyl sulphate (SDS), 30% formamide. Hybridisation was performed in the same buffer for 18 hours at 42°C, including 0.5-1.10^6 cpm ^{32}P-labelled probe per ml of hybridisation buffer. Filters were washed at 55°C in 5x SSPE (2x 15 minutes) and 1x SSPE (2x 15 minutes) and exposed to Kodak XAR film for 1-3 days. Positive clones were plaque purified using standard techniques, and the Bluescript plasmid (Stratagene) "rescued" according to manufacturer's instructions. cDNA clones were sequenced on both strands by standard techniques using Sequenase II enzyme (United

States Biochemicals). The nucleotide sequence of the cDNA encoding the human $GABA_A$ receptor α_2 subunit, together with the deduced amino acid sequence corresponding thereto, is shown in Fig. 2 of the accompanying drawings.

c) <u>Isolation of cDNA encoding human α_3 subunit</u>

A bovine α_3 cDNA (obtained from E. Barnard, Molecular Neurobiology, University of Cambridge, Hills Road, Cambridge; Levitan <u>et</u> <u>al</u>., <u>Nature</u>, 1988, <u>335</u>, 76) was labelled to high specific activity with ^{32}P by random priming and used as a probe. Library filters were prehybridised for 3-6 hours at 55°C in 5x SSPE, 5x Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and hybridised for 18 hours, 55°C in the same buffer, containing 0.5-1x 10^6 cpm/ml of ^{32}P-labelled bovine α_3 cDNA as probe. Filters were washed and exposed to X-ray film as described above; cDNA clones were rescued and sequenced as described above. The longest α_3 cDNA clone was missing in approximately 100 bp of the 5' end of the coding region. This was obtained by PCR using as primers an oligonucleotide "anchor" primer derived from the T7 primer sequence of Bluescript vector (5'AGCGCGCGTAATACGACTCACTATAGGGCGAA3') and an oligonucleotide derived from sequence near the 5' end of the truncated α_3 cDNA, containing an internal Hpal site (5'CAGCATGAATTGTTAACCTCATTGTA3'). Oligonucleotides were synthesised on an Applied Biosystems 380B synthesiser. PCR was performed as described above, and a 300bp PCR product obtained which was double digested with Hpal and Kpnl and subcloned into the similarly cut truncated α_3 cDNA to yield a full length human α_3 cDNA. The cDNA was sequenced on both strands as described above. The nucleotide sequence of the cDNA encoding the human $GABA_A$ receptor α_3 subunit, together with the deduced amino acid

sequence corresponding thereto, is shown in Fig. 3 of the accompanying drawings.

d) Isolation of cDNA encoding human α_5 subunit

A rat α_5 cDNA obtained by polymerase chain reaction (PCR) was used as a probe to screen the cDNA library. For PCR, sequences of the oligonucleotide primers were taken from the published α_5 sequences (Khrestchatisky et al., Neuron, 1989, 3, 745) and incorporated a Hind III site for subcloning purposes: 5' ATTATTCAAGCTTGCCATGGACAATGGAATGCTC3' (bp114-148); 5'GGTTTCCAGCTTACTTTGGAGAGGTAGC3' (bp1507-1535). PCR and subcloning of the PCR product into Bluescript SK-vector (Stratagene) for analysis was performed as described elsewhere (Whiting et al., Proc. Natl. Acad. Sci. USA, 1990, 87, 9966) except that rat brain cDNA was used as template. The rat α_5 cDNA was labelled with ^{32}P and used to screen the human hippocampal cDNA library, and positive α_5 clones rescued and sequenced as described for α_2 above. The nucleotide sequence of the cDNA encoding the human $GABA_A$ receptor α_5 subunit, together with the deduced amino acid sequence corresponding thereto, is shown in Fig. 4 of the accompanying drawings.

e) Isolation of cDNA encoding human α_6 subunit

A rat α_6 cDNA obtained by PCR was used as a probe to screen the cDNA library. PCR was performed as described above for α_5, using oligonucleotide primers derived from the published rat α_6 sequence (Luddens et al., Nature, 1990, 346, 648) incorporating an EcoRI site for subcloning purposes: 5'GAGGAAGAATTCAGGAGGGTGACCT3' (bp48-72); 5'GAAAATAACGAATTCCAGTGTCCAGCTTT3' (bp1376-1404). The rat α_6 cDNA clone isolated by PCR was labelled with ^{32}P and used to screen a human cerebellum cDNA library, as described above for α_2. Positive α_6 clones were purified, rescued and sequenced as described above. None of the cDNAs contained a complete coding

region. To obtain a full length cDNA 3 clones were joined together using convenient restriction sites. The nucleotide sequence of the cDNA encoding the human $GABA_A$ receptor α_6 subunit, together with the deduced amino acid sequence corresponding thereto, is shown in Fig. 5 of the accompanying drawings.

f) Isolation of cDNA encoding human β_2 subunit

Human β_2 cDNA was isolated using as a probe a short human β_2 cDNA obtained by PCR. PCR was performed as described above (except that the human cerebellum cDNA library was used as template), using oligonucleotide primers derived from the published rat β_2 sequence (Ymer et al., EMBO J., 1989, 8, 1665), incorporating EcoRI sites for subcloning purposes: 5' CAAAAGAATTCAGCTGAGAAAGCTGCTAATGC3' (bp1088-1119); 5' TCAGGCGAATTCTCTTTTGTGCCACATGTCGTTC3' (bp1331-1364). The human β_2 clone obtained by PCR was radiolabelled with ^{32}P and used to screen a human hippocampal cDNA library, as described above for α_2. The largest cDNA clone obtained lacked the 5' 500bp of the coding region of the β_2 subunit. This was obtained by PCR using as primers an oligonucleotide "anchor" primer derived from the T7 primer sequence of the Bluescript vector (5' AGCGCGCGTAATACGACTCACTATAGGGCGAA3'), and an oligonucleotide derived from sequence near the 5' end of the truncated β_2 cDNA, containing a Kpnl site (5' CATCCAGTGGGTACCTCCTTAGGT3'). PCR was performed as described above, and a 700bp PCR product obtained which was digested with kpnl and subcloned into the truncated cDNA clone (also Kpnl digested) to yield a full length human β_2 cDNA. The nucleotide sequence of the cDNA encoding the human $GABA_A$ receptor β_2 subunit, together with the deduced amino acid sequence corresponding thereto, is shown in Fig. 6 of the accompanying drawings.

EXAMPLE 4

PREPARATION OF STABLY TRANSFECTED CELLS EXPRESSING $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_3\gamma_{2S}$ AND $\alpha_5\beta_3\gamma_{2S}$ SUBUNIT COMBINATIONS OF THE HUMAN GABA$_A$ RECEPTOR

Isolation and sequence of human α_2 and α_5 cDNAs have been described in Example 3. The sequence of human α_1 cDNA has been published previously by Schofield et al., FEBS Lett., 1989, 244, 361. It differs from the bovine sequence at a single amino acid (trp95 in bovine α_1; arg in human α_1). To create a human α_1 cDNA the bovine sequence was converted to the human by site directed mutagenesis of amino acid 95 with the oligonucleotide 5'GCAATGAAAATCCGGACTGGCAT3', using methods described elsewhere (K. Wafford and P. Whiting, FEBS Lett., 1992, 313, 113-117). The sequence of human γ_2 has been published previously by Pritchett et al., Nature, 1989, 338, 582. A human γ_2 cDNA was isolated by PCR using conditions described elsewhere (Whiting et al., Proc. Natl. Acad. Sci. USA, 1990, 87, 9966-9970), using human hippocampal cDNA library as template and oligonucleotide primers derived from the 5' and 3' untranslated regions of the published γ_2 sequence, incorporating a Hind III restriction site: 5'GGGAGGGAAGCTTCTGCAACCAAGAGGC3', 5'ACCACATAGAAGCTTATTTAAGTGGAC3'. Sequencing indicated that the form of γ_2 used is the short form, γ_{2S}, lacking the 24 bp insert in the putative cytoplasmic loop region (Whiting et al., Proc. Natl. Acad. Sci. USA, 1990, 87, 9966-9970). The sequence of human β_3 has been published by Wagstaff et al., Am. J. Hum. Genet., 1991, 41, 330-337. A human β_3 cDNA was isolated by screening a human foetal brain cDNA library (see Example 3) with a short human β_3 cDNA probe encoding the putative cytoplasmic loop domain which had been obtained using PCR.

Human α_1, α_2, α_5, β_3 and γ_{2S} cDNAs were subcloned into the eukaryotic expression vector pMSGneo (see Example 1) using standard techniques (cf. Maniatis et al. in Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, New York, 2nd Edition, 1989) and stable cell lines expressing human $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_3\gamma_{2S}$ and $\alpha_5\beta_3\gamma_{2S}$ $GABA_A$ receptors were established as described in Example 1.

EXAMPLE 5

PREPARATION OF STABLY TRANSFECTED CELLS EXPRESSING $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_3\beta_3\gamma_{2S}$ AND $\alpha_6\beta_3\gamma_{2S}$ SUBUNIT COMBINATIONS OF THE HUMAN $GABA_A$ RECEPTOR

Isolation of α_3 and α_6 cDNAs is as described in Example 3, and isolation of α_1, β_3 and γ_{2S} cDNAs is as described above in Example 4. Human β_1 subunit cDNA was isolated by PCR from human brain cDNA as described above. Oligonucleotide primers used for the PCR were derived from the published human β_1 sequence (Schofield et al., FEBS Lett., 1989, 244, 361-364), 5' and 3' untranslated regions incorporating Hind III restriction enzyme sites for subcloning:-
5'TAATCAAGCTTAGTAATGTGGACAGTACAAAAT3' and
5'AAATGGAAGCTTTAGAACAGACCTCAGTGTACA3'. Human α_1, α_3, α_6, β_1, β_2, β_3 and γ_{2S} cDNAs were subcloned into the eukaryotic expression vector pMSGneo (see Example 1) using standard techniques (cf. Maniatis et al. in Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, New York, 2nd Edition, 1989) and stable cell lines expressing human $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_3\beta_3\gamma_{2S}$ and $\alpha_6\beta_3\gamma_{2S}$ $GABA_A$ receptors were established as described in Example 1.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Merck Sharp & Dohme Limited
(B) STREET: Hertford Road
(C) CITY: Hoddesdon
(D) STATE: Hertfordshire
(E) COUNTRY: England
(F) POSTAL CODE (ZIP): EN11 9BU

(ii) TITLE OF INVENTION: Stably transfected cell lines expressing GABA-A receptors

(iii) NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2310 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 298..1683

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CTTGCTGGCT TTGAACGCGT GGCGTGGCAG ATATTTCAGA AAGCTTCAAG AACAAGCTGG 240

AGAAGGGAAG AGTTATTCCT CCATATTCAC CTGCTTCAAC TACTATTCTT ATTGGGA     297

ATG GAC AAT GGA ATG TTC TCT GGT TTT ATC ATG ATC AAA AAC CTC CTT   345
Met Asp Asn Gly Met Phe Ser Gly Phe Ile Met Ile Lys Asn Leu Leu
  1               5                  10                  15

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Leu Phe Cys Ile Ser Met Asn Leu Ser Ser His Phe Gly Phe Ser Gln
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ATG CCA ACC AGT TCA GTG AAA GAT GAG ACC AAT GAC AAC ATC ACG ATA   441
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TTT ACC AGG ATC TTG GAT GGG CTC TTG GAT GGC TAC GAC AAC AGA CTT   489
Phe Thr Arg Ile Leu Asp Gly Leu Leu Asp Gly Tyr Asp Asn Arg Leu
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CGG CCC GGG CTG GGA GAG CGC ATC ACT CAG GTG AGG ACC GAC ATC TAC   537
Arg Pro Gly Leu Gly Glu Arg Ile Thr Gln Val Arg Thr Asp Ile Tyr
 65                  70                  75                  80
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Val Thr Ser Phe Gly Pro Val Ser Asp Thr Glu Met Glu Tyr Thr Ile
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GAC GTG TTT TTC CGA CAA AGC TGG AAA GAT GAA AGG CTT CGG TTT AAG   633
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            100                 105                 110

GGG CCC ATG CAG CGC CTC CCT CTC AAC AAC CTC CTT GCC AGC AAG ATC   681
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        115                 120                 125

TGG ACC CCA GAC ACG TTC TTC CAC AAC GGG AAG AAG TCC ATC GCT CAC   729
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    130                 135                 140

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Asn Met Thr Thr Pro Asn Lys Leu Leu Arg Leu Glu Asp Asp Gly Thr
145                 150                 155                 160

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Leu Leu Tyr Thr Met Arg Leu Thr Ile Ser Ala Glu Cys Pro Met Gln
                165                 170                 175

CTT GAG GAC TTC CCG ATG GAT GCG CAC GCT TGC CCT CTG AAA TTT GGC   873
Leu Glu Asp Phe Pro Met Asp Ala His Ala Cys Pro Leu Lys Phe Gly
            180                 185                 190

AGC TAT GCG TAC CCT AAT TCT GAA GTC GTT TAC GTC TGG ACC AAC GGC   921
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        195                 200                 205

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Ser Thr Lys Ser Val Val Val Ala Glu Asp Gly Ser Arg Leu Asn Gln
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TAC CAC CTG ATG GGG CAG ACG GTG GGC ACT GAG AAC ATC AGC ACC AGC  1017
Tyr His Leu Met Gly Gln Thr Val Gly Thr Glu Asn Ile Ser Thr Ser
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ACA GGC GAA TAC ACA ATC ATG ACA GCT CAC TTC CAC CTG AAA AGG AAG  1065
Thr Gly Glu Tyr Thr Ile Met Thr Ala His Phe His Leu Lys Arg Lys
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ATT GGC TAC TTT GTC ATC CAG ACC TAC CTT CCC TGC ATA ATG ACC GTG  1113
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ATC TTA TCA CAG GTG TCC TTT TGG CTG AAC CGG GAA TCA GTC CCA GCC  1161
Ile Leu Ser Gln Val Ser Phe Trp Leu Asn Arg Glu Ser Val Pro Ala
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Arg Thr Val Phe Gly Val Thr Thr Val Leu Thr Met Thr Thr Leu Ser
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ATC AGC GCC AGG AAC TCT CTG CCC AAA GTG GCC TAC GCC ACC GCC ATG  1257
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305                 310                 315                 320

GAC TGG TTC ATA GCT GTG TGC TAT GCC TTC GTC TTC TCG GCG CTG ATA  1305
Asp Trp Phe Ile Ala Val Cys Tyr Ala Phe Val Phe Ser Ala Leu Ile
            325                 330                 335

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        340                 345                 350

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1803

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1863

CCAAGATGTC CCATTGATAA TTCGAGCAAA CAACTTTCTG GAAAAACAGG ATACGATGAC
1923

TGACACTCAG ATGCCCAGTA TCATACGTTG ATAGTTTACA AACAAGATAC GTATATTTTT

1983

AACTGCTTCA AGTGTTACCT AACAATGTTT TTTATACTTC AAATGTCATT TCATACAAAT 2043

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GCGAGAAACA GAGATCATAA AGAGCACGTT TTCCATTATG AGGAAACTTG GACATTTATG 2163

TACAAAATGA ATTGCCTTTG ATAATTCTTA CTGTTCTGAA ATTAGGAAAG TACTTGCATG 2223

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CATCATATGT TAGATACACA AAATATT 2310

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Val Thr Ser Phe Gly Pro Val Ser Asp Thr Glu Met Glu Tyr Thr Ile
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Gly Pro Met Gln Arg Leu Pro Leu Asn Asn Leu Leu Ala Ser Lys Ile
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130 135 140

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Phe Pro Val Leu Phe Gly Thr Phe Asn Leu Val Tyr Trp Ala Thr Tyr
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 27..1385

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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30 35 40

GGC TAT GAC AAT CGG CTG CGG CCG GGA TTT GGA GGT GCT GTC ACT GAA 197
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75 80 85

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110 115 120

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Lys Lys Ser Ile Ala His Asn Met Thr Thr Pro Asn Lys Leu Phe Arg
125 130 135

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140 145 150

GCT GAC TGT CCC ATG AGG CTG GTT AAC TTT CCT ATG GAT GGG CAT GCT 533
Ala Asp Cys Pro Met Arg Leu Val Asn Phe Pro Met Asp Gly His Ala
155 160 165

TGT CCA CTC AAG TTT GGG AGC TAT GCT TAT CCC AAA AGT GAA ATC ATA 581
Cys Pro Leu Lys Phe Gly Ser Tyr Ala Tyr Pro Lys Ser Glu Ile Ile
170 175 180 185

TAT ACG TGG AAA AAA GGA CCA CTT TAC TCA GTA GAA GTC CCA GAA GAA 629
Tyr Thr Trp Lys Lys Gly Pro Leu Tyr Ser Val Glu Val Pro Glu Glu
190 195 200

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205 210 215

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220 225 230

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285 290 295

TCA TAT GCC ACT GCC ATG GAT TGG TTC ATA GCT GTT TGC TTT GCA TTC 965
Ser Tyr Ala Thr Ala Met Asp Trp Phe Ile Ala Val Cys Phe Ala Phe
300 305 310

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GTC TTC TCT GCT CTT ATC GAG TTC GCA GCT GTC AAC TAC TTT ACC AAT  1013
Val Phe Ser Ala Leu Ile Glu Phe Ala Ala Val Asn Tyr Phe Thr Asn
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ACA GTG ACA ATA TCA AAA GCT ACT GAA CCT TTG GAA GCT GAG ATT GTT  1109
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                350                 355                 360

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Leu His Pro Asp Ser Lys Tyr His Leu Lys Lys Arg Ile Thr Ser Leu
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Ser Leu Pro Ile Val Ser Ser Ser Glu Ala Asn Lys Val Leu Thr Arg
        380                 385                 390

GCG CCC ATC TTA CAA TCA ACA CCT GTC ACA CCC CCA CCA CTC CCG CCA  1253
Ala Pro Ile Leu Gln Ser Thr Pro Val Thr Pro Pro Pro Leu Pro Pro
    395                 400                 405

GCC TTT GGA GGC ACC AGT AAA ATA GAC CAG TAT TCT CGA ATT CTC TTC  1301
Ala Phe Gly Gly Thr Ser Lys Ile Asp Gln Tyr Ser Arg Ile Leu Phe
410                 415                 420                 425

CCA GTT GCA TTT GCA GGA TTC AAC CTT GTG TAC TGG GTA GTT TAT CTT  1349
Pro Val Ala Phe Ala Gly Phe Asn Leu Val Tyr Trp Val Val Tyr Leu
                430                 435                 440

TCC AAA GAT ACA ATG GAA GTG AGT AGC AGT GTT GAA TAGCTTTTCC       1395
Ser Lys Asp Thr Met Glu Val Ser Ser Ser Val Glu
            445                 450

AGGACAACCT GAA                                                   1408
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 453 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Ala Ser Ser Leu Pro Trp Leu Cys Ile Ile Leu Trp Leu Glu Asn
1 5 10 15

Ala Leu Gly Lys Leu Glu Val Glu Gly Asn Phe Tyr Ser Glu Asn Val
20 25 30

Ser Arg Ile Leu Asp Asn Leu Leu Glu Gly Tyr Asp Asn Arg Leu Arg
35 40 45

Pro Gly Phe Gly Gly Ala Val Thr Glu Val Lys Thr Asp Ile Tyr Val
50 55 60

Thr Ser Phe Gly Pro Val Ser Asp Val Glu Met Glu Tyr Thr Met Asp
65 70 75 80

Val Phe Phe Arg Gln Thr Trp Thr Asp Glu Arg Leu Lys Phe Gly Gly
85 90 95

Pro Thr Glu Ile Leu Ser Leu Asn Asn Leu Met Val Ser Lys Ile Trp
100 105 110

Thr Pro Asp Thr Phe Phe Arg Asn Gly Lys Lys Ser Ile Ala His Asn
115 120 125

Met Thr Thr Pro Asn Lys Leu Phe Arg Ile Met Gln Asn Gly Thr Ile
130 135 140

Leu Tyr Thr Met Arg Leu Thr Ile Asn Ala Asp Cys Pro Met Arg Leu
145 150 155 160

Val Asn Phe Pro Met Asp Gly His Ala Cys Pro Leu Lys Phe Gly Ser
165 170 175

Tyr Ala Tyr Pro Lys Ser Glu Ile Ile Tyr Thr Trp Lys Lys Gly Pro
180 185 190

Leu Tyr Ser Val Glu Val Pro Glu Glu Ser Ser Ser Leu Leu Gln Tyr
195 200 205

Asp Leu Ile Gly Gln Thr Val Ser Ser Glu Thr Ile Lys Ser Asn Thr
210 215 220

Gly Glu Tyr Val Ile Met Thr Val Tyr Phe His Leu Gln Arg Lys Met
225 230 235 240

Gly Tyr Phe Met Ile Gln Ile Tyr Thr Pro Cys Ile Met Thr Val Ile
245 250 255

Leu Ser Gln Val Ser Phe Trp Ile Asn Lys Glu Ser Val Pro Ala Arg
260 265 270

Thr Val Leu Gly Ile Thr Thr Val Leu Thr Met Thr Thr Leu Ser Ile
275 280 285

Ser Ala Arg His Ser Leu Pro Lys Val Ser Tyr Ala Thr Ala Met Asp
290 295 300

Trp Phe Ile Ala Val Cys Phe Ala Phe Val Phe Ser Ala Leu Ile Glu
305 310 315 320

Phe Ala Ala Val Asn Tyr Phe Thr Asn Leu Gln Thr Gln Lys Ala Lys
325 330 335

Arg Lys Ala Gln Phe Ala Ala Pro Pro Thr Val Thr Ile Ser Lys Ala
340 345 350

Thr Glu Pro Leu Glu Ala Glu Ile Val Leu His Pro Asp Ser Lys Tyr
355 360 365

His Leu Lys Lys Arg Ile Thr Ser Leu Ser Leu Pro Ile Val Ser Ser
370 375 380

Ser Glu Ala Asn Lys Val Leu Thr Arg Ala Pro Ile Leu Gln Ser Thr
385 390 395 400

Pro Val Thr Pro Pro Pro Leu Pro Pro Ala Phe Gly Gly Thr Ser Lys
405 410 415

Ile Asp Gln Tyr Ser Arg Ile Leu Phe Pro Val Ala Phe Ala Gly Phe
420 425 430

Asn Leu Val Tyr Trp Val Val Tyr Leu Ser Lys Asp Thr Met Glu Val
435 440 445

Ser Ser Ser Val Glu
450

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1866 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 225..1646

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
GAATTCCGCG CGGGGAAGGG AAGAAGAGGA CGAGGTGGCG CAGAGACCGC GGGAGAACAC  60

AGTGCCTCCG GAGGAAATCT GCTCGGTCCC CGGCAGCCGC GCTTCCCCTT TGATGTTTTG 120

GTACGCCGTG GCCATGCGCC TCACATTAGA ATTACTGCAC TGGGCAGACT AAGTTGGATC 180

TCCTCTCTTC AGTGAAACCC TCAATTCCAT CAAAAACTAA AGGG ATG TGG AGA GTG  236
                                                 Met Trp Arg Val
                                                   1

CGG AAA AGG GGC TAC TTT GGG ATT TGG TCC TTC CCC TTA ATA ATC GCC  284
Arg Lys Arg Gly Tyr Phe Gly Ile Trp Ser Phe Pro Leu Ile Ile Ala
  5                  10                  15                  20

GCT GTC TGT GCG CAG AGT GTC AAT GAC CCT AGT AAT ATG TCG CTG GTT  332
Ala Val Cys Ala Gln Ser Val Asn Asp Pro Ser Asn Met Ser Leu Val
                 25                  30                  35

AAA GAG ACG GTG GAT AGA CTC CTG AAA GGC TAT GAC ATT CGT CTG AGA  380
Lys Glu Thr Val Asp Arg Leu Leu Lys Gly Tyr Asp Ile Arg Leu Arg
             40                  45                  50

CCA GAT TTT GGA GGT CCC CCC GTG GCT GTG GGG ATG AAC ATT GAC ATT  428
Pro Asp Phe Gly Gly Pro Pro Val Ala Val Gly Met Asn Ile Asp Ile
         55                  60                  65

GCC AGC ATC GAT ATG GTT TCT GAA GTC AAT ATG GAT TAT ACC TTG ACA  476
Ala Ser Ile Asp Met Val Ser Glu Val Asn Met Asp Tyr Thr Leu Thr
     70                  75                  80
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ATG TAC TTT CAA CAA GCC TGG AGA GAT AAG AGG CTG TCC TAT AAT GTA   524
Met Tyr Phe Gln Gln Ala Trp Arg Asp Lys Arg Leu Ser Tyr Asn Val
 85                  90                  95                 100

ATA CCT TTA AAC TTG ACT CTG GAC AAC AGA GTG GCA GAC CAG CTC TGG   572
Ile Pro Leu Asn Leu Thr Leu Asp Asn Arg Val Ala Asp Gln Leu Trp
                105                 110                 115

GTG CCT GAT ACC TAT TTC CTG AAC GAT AAG AAG TCA TTT GTG CAC GGA   620
Val Pro Asp Thr Tyr Phe Leu Asn Asp Lys Lys Ser Phe Val His Gly
            120                 125                 130

GTG ACT GTT AAG AAC CGC ATG ATT CGC CTG CAT CCT GAT GGC ACC GTC   668
Val Thr Val Lys Asn Arg Met Ile Arg Leu His Pro Asp Gly Thr Val
        135                 140                 145

CTT TAT GGA CTC AGA ATC ACA ACC ACA GCT GCC TGC ATG ATG GAC CTA   716
Leu Tyr Gly Leu Arg Ile Thr Thr Thr Ala Ala Cys Met Met Asp Leu
    150                 155                 160

AGG AGG TAC CCA CTG GAT GAA CAA AAC TGC ACC TTG GAA ATT GAG AGC   764
Arg Arg Tyr Pro Leu Asp Glu Gln Asn Cys Thr Leu Glu Ile Glu Ser
165                 170                 175                 180

TAT GGA TAC ACA ACT GAT GAC ATT GAG TTT TAC TGG CGT GGC GAT GAT   812
Tyr Gly Tyr Thr Thr Asp Asp Ile Glu Phe Tyr Trp Arg Gly Asp Asp
                185                 190                 195

AAT GCA GTA ACA GGA GTA ACG AAA ATT GAA CTT CCA CAG TTC TCT ATT   860
Asn Ala Val Thr Gly Val Thr Lys Ile Glu Leu Pro Gln Phe Ser Ile
            200                 205                 210

GTA GAT TAC AAA CTT ATC ACC AAG AAG GTT GTT TTT TCC ACA GGT TCC   908
Val Asp Tyr Lys Leu Ile Thr Lys Lys Val Val Phe Ser Thr Gly Ser
        215                 220                 225
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TAT CCC AGG TTA TCC CTC AGC TTT AAG CTT AAG AGA AAC ATT GGC TAC   956
Tyr Pro Arg Leu Ser Leu Ser Phe Lys Leu Lys Arg Asn Ile Gly Tyr
    230                 235                 240

TTT ATC CTG CAA ACA TAC ATG CCT TCC ATC CTG ATT ACC ATC CTC TCC  1004
Phe Ile Leu Gln Thr Tyr Met Pro Ser Ile Leu Ile Thr Ile Leu Ser
245                 250                 255                 260

TGG GTC TCC TTC TGG ATT AAT TAC GAT GCT TCA GCT GCA AGG GTG GCA  1052
Trp Val Ser Phe Trp Ile Asn Tyr Asp Ala Ser Ala Ala Arg Val Ala
                265                 270                 275

TTA GGA ATC ACA ACT GTC CTC ACA ATG ACC ACA ATC AAC ACC CAC CTC  1100
Leu Gly Ile Thr Thr Val Leu Thr Met Thr Thr Ile Asn Thr His Leu
            280                 285                 290

CGG GAA ACT CTC CCT AAA ATC CCC TAT GTG AAG GCC ATT GAC ATG TAC  1148
Arg Glu Thr Leu Pro Lys Ile Pro Tyr Val Lys Ala Ile Asp Met Tyr
        295                 300                 305

CTG ATG GGG TGC TTT GTC TTC GTT TTC ATG GCC CTT CTG GAA TAT GCC  1196
Leu Met Gly Cys Phe Val Phe Val Phe Met Ala Leu Leu Glu Tyr Ala
    310                 315                 320

CTA GTC AAC TAC ATC TTC TTT GGG AGG GGG CCC CAA CGC CAA AAG AAA  1244
Leu Val Asn Tyr Ile Phe Phe Gly Arg Gly Pro Gln Arg Gln Lys Lys
325                 330                 335                 340

GCA GCT GAG AAG GCT GCC AGT GCC AAC AAT GAG AAG ATG CGC CTG GAT  1292
Ala Ala Glu Lys Ala Ala Ser Ala Asn Asn Glu Lys Met Arg Leu Asp
                345                 350                 355

GTC AAC AAG ATG GAC CCC CAT GAG AAC ATC TTA CTG AGC ACT CTC GAG  1340
Val Asn Lys Met Asp Pro His Glu Asn Ile Leu Leu Ser Thr Leu Glu
            360                 365                 370
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ATA AAA AAT GAA ATG GCC ACA TCT GAG GCT GTG ATG GGA CTT GGA GAC 1388
Ile Lys Asn Glu Met Ala Thr Ser Glu Ala Val Met Gly Leu Gly Asp
375 380 385

CCC AGA AGC ACA ATG CTA GCC TAT GAT GCC TCC AGC ATC CAG TAT CGG 1436
Pro Arg Ser Thr Met Leu Ala Tyr Asp Ala Ser Ser Ile Gln Tyr Arg
390 395 400

AAA GCT GGG TTG CCC AGG CAT AGT TTT GGC CGA AAT GCT CTG GAA CGA 1484
Lys Ala Gly Leu Pro Arg His Ser Phe Gly Arg Asn Ala Leu Glu Arg
405 410 415 420

CAT GTG GCG CAA AAG AAA AGT CGC CTG AGG AGA CGC GCC TCC CAA CTG 1532
His Val Ala Gln Lys Lys Ser Arg Leu Arg Arg Arg Ala Ser Gln Leu
425 430 435

AAA ATC ACC ATC CCT GAC TTG ACT GAT GTG AAT GCC ATA GAT CGG TGG 1580
Lys Ile Thr Ile Pro Asp Leu Thr Asp Val Asn Ala Ile Asp Arg Trp
440 445 450

TCC CGC ATA TTC TTC CCA GTG GTT TTT TCC TTC TTC AAC ATC GTC TAT 1628
Ser Arg Ile Phe Phe Pro Val Val Phe Ser Phe Phe Asn Ile Val Tyr
455 460 465

TGG CTT TAT TAT GTG AAC TAAAACATGG CCTCCCACTG GAAGCAAGGA 1676
Trp Leu Tyr Tyr Val Asn
470

CTAGATTCCT CCTCAAACCA GTTGTACAGC CTGATGTAGG ACTTGGAAAA CACATCAATC
1736

CAGGACAAAA GTGACGCTAA AATACCTTAG TTGCTGGCCT ATCCTGTGGT CCATTTCATA
1796

CCATTTGGGT TGCTTCTGCT AAGTAATGAA TACACTAAGG TCCTTGTGGT TTTCCAGTTA
1856

AAACGCAAGT 1866

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 474 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Trp Arg Val Arg Lys Arg Gly Tyr Phe Gly Ile Trp Ser Phe Pro
1 5 10 15

Leu Ile Ile Ala Ala Val Cys Ala Gln Ser Val Asn Asp Pro Ser Asn
20 25 30

Met Ser Leu Val Lys Glu Thr Val Asp Arg Leu Leu Lys Gly Tyr Asp
35 40 45

Ile Arg Leu Arg Pro Asp Phe Gly Gly Pro Pro Val Ala Val Gly Met
50 55 60

Asn Ile Asp Ile Ala Ser Ile Asp Met Val Ser Glu Val Asn Met Asp
65 70 75 80

Tyr Thr Leu Thr Met Tyr Phe Gln Gln Ala Trp Arg Asp Lys Arg Leu
85 90 95

Ser Tyr Asn Val Ile Pro Leu Asn Leu Thr Leu Asp Asn Arg Val Ala
100 105 110

Asp Gln Leu Trp Val Pro Asp Thr Tyr Phe Leu Asn Asp Lys Lys Ser
115 120 125

Phe Val His Gly Val Thr Val Lys Asn Arg Met Ile Arg Leu His Pro
130 135 140

Asp Gly Thr Val Leu Tyr Gly Leu Arg Ile Thr Thr Thr Ala Ala Cys
145 150 155 160

Met Met Asp Leu Arg Arg Tyr Pro Leu Asp Glu Gln Asn Cys Thr Leu
165 170 175

Glu Ile Glu Ser Tyr Gly Tyr Thr Thr Asp Asp Ile Glu Phe Tyr Trp
180 185 190

Arg Gly Asp Asp Asn Ala Val Thr Gly Val Thr Lys Ile Glu Leu Pro
195 200 205

Gln Phe Ser Ile Val Asp Tyr Lys Leu Ile Thr Lys Lys Val Val Phe
210 215 220

Ser Thr Gly Ser Tyr Pro Arg Leu Ser Leu Ser Phe Lys Leu Lys Arg
225 230 235 240

Asn Ile Gly Tyr Phe Ile Leu Gln Thr Tyr Met Pro Ser Ile Leu Ile
245 250 255

Thr Ile Leu Ser Trp Val Ser Phe Trp Ile Asn Tyr Asp Ala Ser Ala
260 265 270

Ala Arg Val Ala Leu Gly Ile Thr Thr Val Leu Thr Met Thr Thr Ile
275 280 285

Asn Thr His Leu Arg Glu Thr Leu Pro Lys Ile Pro Tyr Val Lys Ala
290 295 300

Ile Asp Met Tyr Leu Met Gly Cys Phe Val Phe Val Phe Met Ala Leu
305 310 315 320

Leu Glu Tyr Ala Leu Val Asn Tyr Ile Phe Phe Gly Arg Gly Pro Gln
325 330 335

Arg Gln Lys Lys Ala Ala Glu Lys Ala Ala Ser Ala Asn Asn Glu Lys
340 345 350

Met Arg Leu Asp Val Asn Lys Met Asp Pro His Glu Asn Ile Leu Leu
355 360 365

Ser Thr Leu Glu Ile Lys Asn Glu Met Ala Thr Ser Glu Ala Val Met
370 375 380

Gly Leu Gly Asp Pro Arg Ser Thr Met Leu Ala Tyr Asp Ala Ser Ser
385 390 395 400

Ile Gln Tyr Arg Lys Ala Gly Leu Pro Arg His Ser Phe Gly Arg Asn
405 410 415

Ala Leu Glu Arg His Val Ala Gln Lys Lys Ser Arg Leu Arg Arg Arg
420 425 430

Ala Ser Gln Leu Lys Ile Thr Ile Pro Asp Leu Thr Asp Val Asn Ala
435 440 445

Ile Asp Arg Trp Ser Arg Ile Phe Phe Pro Val Val Phe Ser Phe Phe
450 455 460

Asn Ile Val Tyr Trp Leu Tyr Tyr Val Asn
465 470

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2189 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 214..1566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCTAGCGCTC CTCTCCGGCT TCCACCAGCC CATCGCTCCA CGCTCTCTTG GCTGCTGCAG 60

TCTCGGTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC 120

TCTCTCTCTC TCTCTCCCAA GTTTCCTATC TCGTCAAGAT CAGGGCAAAA GAAGAAAACA 180

CCGAATTCTG CTTGCCGTTT CAGAGCGGCG GTG ATG AAG ACA AAA TTG AAC ATC 234
Met Lys Thr Lys Leu Asn Ile
1 5

TAC AAC ATC GAG TTC CTG CTT TTT GTT TTC TTG GTG TGG GAC CCT GCC 282
Tyr Asn Ile Glu Phe Leu Leu Phe Val Phe Leu Val Trp Asp Pro Ala
10 15 20

AGG TTG GTG CTG GCT AAC ATC CAA GAA GAT GAG GCT AAA AAT AAC ATT 330
Arg Leu Val Leu Ala Asn Ile Gln Glu Asp Glu Ala Lys Asn Asn Ile
25 30 35

ACC ATC TTT ACG AGA ATT CTT GAC AGA CTT CTG GAT GGT TAC GAT AAT 378
Thr Ile Phe Thr Arg Ile Leu Asp Arg Leu Leu Asp Gly Tyr Asp Asn
40 45 50 55

CGG CTT AGA CCA GGA CTG GGA GAC AGT ATT ACT GAA GTC TTC ACT AAC 426
Arg Leu Arg Pro Gly Leu Gly Asp Ser Ile Thr Glu Val Phe Thr Asn
60 65 70

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ATC TAC GTG ACC AGT TTT GGC CCT GTC TCA GAT ACA GAT ATG GAA TAT   474
Ile Tyr Val Thr Ser Phe Gly Pro Val Ser Asp Thr Asp Met Glu Tyr
            75                  80                  85

ACA ATT GAT GTT TTC TTT CGA CAA AAA TGG AAA GAT GAA CGT TTA AAA   522
Thr Ile Asp Val Phe Phe Arg Gln Lys Trp Lys Asp Glu Arg Leu Lys
        90                  95                  100

TTT AAA GGT CCT ATG AAT ATC CTT CGA CTA AAC AAT TTA ATG GCT AGC   570
Phe Lys Gly Pro Met Asn Ile Leu Arg Leu Asn Asn Leu Met Ala Ser
    105                 110                 115

AAA ATC TGG ACT CCA GAT ACC TTT TTT CAC AAT GGG AAG AAA TCA GTA   618
Lys Ile Trp Thr Pro Asp Thr Phe Phe His Asn Gly Lys Lys Ser Val
120                 125                 130                 135

GCT CAT AAT ATG ACA ATG CCA AAT AAG TTG CTT CGA ATT CAG GAT GAT   666
Ala His Asn Met Thr Met Pro Asn Lys Leu Leu Arg Ile Gln Asp Asp
                140                 145                 150

GGG ACT CTG CTG TAT ACC ATG AGG CTT ACA GTT CAA GCT GAA TGC CCA   714
Gly Thr Leu Leu Tyr Thr Met Arg Leu Thr Val Gln Ala Glu Cys Pro
            155                 160                 165

ATG CAC TTG GAG GAT TTC CCA ATG GAT GCT CAT TCA TGT CCT CTG AAA   762
Met His Leu Glu Asp Phe Pro Met Asp Ala His Ser Cys Pro Leu Lys
        170                 175                 180

TTT GGC AGC TAT GCA TAT ACA ACT TCA GAG GTC ACT TAT ATT TGG ACT   810
Phe Gly Ser Tyr Ala Tyr Thr Thr Ser Glu Val Thr Tyr Ile Trp Thr
    185                 190                 195

TAC AAT GCA TCT GAT TCA GTA CAG GTT GCT CCT GAT GGC TCT AGG TTA   858
Tyr Asn Ala Ser Asp Ser Val Gln Val Ala Pro Asp Gly Ser Arg Leu
200                 205                 210                 215
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AAT CAA TAT GAC CTG CTG GGC CAA TCA ATC GGA AAG GAG ACA ATT AAA 906
Asn Gln Tyr Asp Leu Leu Gly Gln Ser Ile Gly Lys Glu Thr Ile Lys
220 225 230

TCC AGT ACA GGT GAA TAT ACT GTA ATG ACA GCT CAT TTC CAC CTG AAA 954
Ser Ser Thr Gly Glu Tyr Thr Val Met Thr Ala His Phe His Leu Lys
235 240 245

AGA AAA ATT GGG TAT TTT GTG ATT CAA ACC TAT CTG CCT TGC ATC ATG 1002
Arg Lys Ile Gly Tyr Phe Val Ile Gln Thr Tyr Leu Pro Cys Ile Met
250 255 260

ACT GTC ATT CTC TCC CAA GTT TCA TTC TGG CTT AAC AGA GAA TCT GTG 1050
Thr Val Ile Leu Ser Gln Val Ser Phe Trp Leu Asn Arg Glu Ser Val
265 270 275

CCT GCA AGA ACT GTG TTT GGA GTA ACA ACT GTC CTA ACA ATG ACA ACT 1098
Pro Ala Arg Thr Val Phe Gly Val Thr Thr Val Leu Thr Met Thr Thr
280 285 290 295

CTA AGC ATC AGT GCT CGG AAT TCT CTC CCC AAA GTG GCT TAT GCA ACT 1146
Leu Ser Ile Ser Ala Arg Asn Ser Leu Pro Lys Val Ala Tyr Ala Thr
300 305 310

GCC ATG GAC TGG TTT ATT GCT GTT TGT TAT GCA TTT GTG TTC TCT GCC 1194
Ala Met Asp Trp Phe Ile Ala Val Cys Tyr Ala Phe Val Phe Ser Ala
315 320 325

CTA ATT GAA TTT GCA ACT GTT AAT TAC TTC ACC AAA AGA GGA TGG ACT 1242
Leu Ile Glu Phe Ala Thr Val Asn Tyr Phe Thr Lys Arg Gly Trp Thr
330 335 340

TGG GAT GGG AAG AGT GTA GTA AAT GAC AAG AAA AAA GAA AAG GCT TCC 1290
Trp Asp Gly Lys Ser Val Val Asn Asp Lys Lys Lys Glu Lys Ala Ser
345 350 355

GTT ATG ATA CAG AAC AAC GCT TAT GCA GTG GCT GTT GCC AAT TAT GCC 1338
Val Met Ile Gln Asn Asn Ala Tyr Ala Val Ala Val Ala Asn Tyr Ala
360 365 370 375

CCG AAT CTT TCA AAA GAT CCA GTT CTC TCC ACC ATC TCC AAG AGT GCA 1386
Pro Asn Leu Ser Lys Asp Pro Val Leu Ser Thr Ile Ser Lys Ser Ala
380 385 390

ACC ACG CCA GAA CCC AAC AAG AAG CCA GAA AAC AAG CCA GCT GAA GCA 1434
Thr Thr Pro Glu Pro Asn Lys Lys Pro Glu Asn Lys Pro Ala Glu Ala
395 400 405

AAG AAA ACT TTC AAC AGT GTT AGC AAA ATT GAC AGA ATG TCC AGA ATA 1482
Lys Lys Thr Phe Asn Ser Val Ser Lys Ile Asp Arg Met Ser Arg Ile
410 415 420

GTT TTT CCA GTT TTG TTT GGT ACC TTT AAT TTA GTT TAC TGG GCT ACA 1530
Val Phe Pro Val Leu Phe Gly Thr Phe Asn Leu Val Tyr Trp Ala Thr
425 430 435

TAT TTA AAC AGA GAA CCT GTA TTA GGG GTC AGT CCT TGAATTGAGA 1576
Tyr Leu Asn Arg Glu Pro Val Leu Gly Val Ser Pro
440 445 450

CCCATGTTAT CTTTGGGATG TATAGCAACA TTAAATTTGG TTTGTTTTGC TATGTACAGT 1636

CTGACTAATA ACTGCTAATT TGTGATCCAA CATGTACAGT ATGTATATAG TGACATAGCT 1696

TACCAGTAGA CCTTTAATGG AGACATGCAT TTGCTAACTC ATGGAACTGC AGACAGAAAG 1756

CACTCCATGC GAAAACAGCC ATTGCCTTTT TTAAAGATTT ACCCTAGGAC CTGATTTAAA 1816

GTGAATTTCA AGTGACCTGA TTAATTTCCT ATTCTTCCAA ATGAGATGAA AATGGGGATC

1876

CTGTACAACC CTTTGTGGAC CCTTTTGGTT TAGCTCTTAA GTAGGGGTAT TTTCTACTGT 1936

TGCTTAATTA TGATGGAAGA TAACATTGTC ATTCCTAGAT GAATCCTTTG AAGTAACAAA 1996

CATTGTATCT GACATCAGCT CTGTTCATGA GTGCTCAGAG TCCCTGCTAA TGTAATTGGA 2056

AGCTTGGTAC ACATAAGAAA AACTAGAGAT TTGAAATCTA GCTATGAATT ACTCTATATA 2116

GTATCTATAG CCATGTACAT ATTACAGCAT GACAAGCTCG AAATAATTAT GAGTCAGCCC 2176

GAAAGATGTT AAT 2189

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Lys Thr Lys Leu Asn Ile Tyr Asn Ile Glu Phe Leu Leu Phe Val
1 5 10 15

Phe Leu Val Trp Asp Pro Ala Arg Leu Val Leu Ala Asn Ile Gln Glu
20 25 30

Asp Glu Ala Lys Asn Asn Ile Thr Ile Phe Thr Arg Ile Leu Asp Arg
35 40 45

Leu Leu Asp Gly Tyr Asp Asn Arg Leu Arg Pro Gly Leu Gly Asp Ser
50 55 60

Ile Thr Glu Val Phe Thr Asn Ile Tyr Val Thr Ser Phe Gly Pro Val
65 70 75 80

Ser Asp Thr Asp Met Glu Tyr Thr Ile Asp Val Phe Phe Arg Gln Lys
85 90 95

Trp Lys Asp Glu Arg Leu Lys Phe Lys Gly Pro Met Asn Ile Leu Arg
100 105 110

Leu Asn Asn Leu Met Ala Ser Lys Ile Trp Thr Pro Asp Thr Phe Phe
115 120 125

His Asn Gly Lys Lys Ser Val Ala His Asn Met Thr Met Pro Asn Lys
130 135 140

Leu Leu Arg Ile Gln Asp Asp Gly Thr Leu Leu Tyr Thr Met Arg Leu
145 150 155 160

Thr Val Gln Ala Glu Cys Pro Met His Leu Glu Asp Phe Pro Met Asp
165 170 175

Ala His Ser Cys Pro Leu Lys Phe Gly Ser Tyr Ala Tyr Thr Thr Ser
180 185 190

Glu Val Thr Tyr Ile Trp Thr Tyr Asn Ala Ser Asp Ser Val Gln Val
195 200 205

Ala Pro Asp Gly Ser Arg Leu Asn Gln Tyr Asp Leu Leu Gly Gln Ser
210 215 220

Ile Gly Lys Glu Thr Ile Lys Ser Ser Thr Gly Glu Tyr Thr Val Met
225 230 235 240

Thr Ala His Phe His Leu Lys Arg Lys Ile Gly Tyr Phe Val Ile Gln
245 250 255

Thr Tyr Leu Pro Cys Ile Met Thr Val Ile Leu Ser Gln Val Ser Phe
260 265 270

Trp Leu Asn Arg Glu Ser Val Pro Ala Arg Thr Val Phe Gly Val Thr
275 280 285

Thr Val Leu Thr Met Thr Thr Leu Ser Ile Ser Ala Arg Asn Ser Leu
290 295 300

Pro Lys Val Ala Tyr Ala Thr Ala Met Asp Trp Phe Ile Ala Val Cys
305 310 315 320

Tyr Ala Phe Val Phe Ser Ala Leu Ile Glu Phe Ala Thr Val Asn Tyr
325 330 335

Phe Thr Lys Arg Gly Trp Thr Trp Asp Gly Lys Ser Val Val Asn Asp
340 345 350

Lys Lys Lys Glu Lys Ala Ser Val Met Ile Gln Asn Asn Ala Tyr Ala
355 360 365

Val Ala Val Ala Asn Tyr Ala Pro Asn Leu Ser Lys Asp Pro Val Leu
370 375 380

Ser Thr Ile Ser Lys Ser Ala Thr Thr Pro Glu Pro Asn Lys Lys Pro
385 390 395 400

Glu Asn Lys Pro Ala Glu Ala Lys Lys Thr Phe Asn Ser Val Ser Lys
405 410 415

Ile Asp Arg Met Ser Arg Ile Val Phe Pro Val Leu Phe Gly Thr Phe
420 425 430

Asn Leu Val Tyr Trp Ala Thr Tyr Leu Asn Arg Glu Pro Val Leu Gly
435 440 445

Val Ser Pro
450

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1638 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 87..1562

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAATTCCCTT GTTTCAGTTC ATTCATCCTT CTCTCCTTTC CGCTCAGACT GTAGAGCTCG 60

GTCTCTCCAA GTTTGTGCCT AAGAAG ATG ATA ATC ACA CAA ACA AGT CAC TGT 113
Met Ile Ile Thr Gln Thr Ser His Cys
1 5

TAC ATG ACC AGC CTT GGG ATT CTT TTC CTG ATT AAT ATT CTC CCT GGA 161
Tyr Met Thr Ser Leu Gly Ile Leu Phe Leu Ile Asn Ile Leu Pro Gly
10 15 20 25

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ACC ACT GGT CAA GGG GAA TCA AGA CGA CAA GAA CCC GGG GAC TTT GTG   209
Thr Thr Gly Gln Gly Glu Ser Arg Arg Gln Glu Pro Gly Asp Phe Val
                30                  35                  40

AAG CAG GAC ATT GGC GGG CTG TCT CCT AAG CAT GCC CCA GAT ATT CCT   257
Lys Gln Asp Ile Gly Gly Leu Ser Pro Lys His Ala Pro Asp Ile Pro
            45                  50                  55

GAT GAC AGC ACT GAC AAC ATC ACT ATC TTC ACC AGA ATC TTG GAT CGT   305
Asp Asp Ser Thr Asp Asn Ile Thr Ile Phe Thr Arg Ile Leu Asp Arg
        60                  65                  70

CTT CTG GAC GGC TAT GAC AAC CGG CTG CGA CCT GGG CTT GGA GAT GCA   353
Leu Leu Asp Gly Tyr Asp Asn Arg Leu Arg Pro Gly Leu Gly Asp Ala
    75                  80                  85

GTG ACT GAA GTG AAG ACT GAC ATC TAC GTG ACC AGT TTT GGC CCT GTG   401
Val Thr Glu Val Lys Thr Asp Ile Tyr Val Thr Ser Phe Gly Pro Val
90                  95                  100                 105

TCA GAC ACT GAC ATG GAG TAC ACT ATT GAT GTA TTT TTT CGG CAG ACA   449
Ser Asp Thr Asp Met Glu Tyr Thr Ile Asp Val Phe Phe Arg Gln Thr
                110                 115                 120

TGG CAT GAT GAA AGA CTG AAA TTT GAT GGC CCC ATG AAG ATC CTT CCA   497
Trp His Asp Glu Arg Leu Lys Phe Asp Gly Pro Met Lys Ile Leu Pro
            125                 130                 135

CTG AAC AAT CTC CTG GCT AGT AAG ATC TGG ACA CCG GAC ACC TTC TTC   545
Leu Asn Asn Leu Leu Ala Ser Lys Ile Trp Thr Pro Asp Thr Phe Phe
        140                 145                 150

CAC AAT GGC AAG AAA TCA GTG GCT CAT AAC ATG ACC ACG CCC AAC AAG   593
His Asn Gly Lys Lys Ser Val Ala His Asn Met Thr Thr Pro Asn Lys
    155                 160                 165
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CTG CTC AGA TTG GTG GAC AAC GGA ACC CTC CTC TAT ACA ATG AGG TTA   641
Leu Leu Arg Leu Val Asp Asn Gly Thr Leu Leu Tyr Thr Met Arg Leu
170                 175                 180                 185

ACA ATT CAT GCT GAG TGT CCC ATG CAT TTG GAA GAT TTT CCC ATG GAT   689
Thr Ile His Ala Glu Cys Pro Met His Leu Glu Asp Phe Pro Met Asp
                190                 195                 200

GTG CAT GCC TGC CCA CTG AAG TTT GGA AGC TAT GCC TAT ACA ACA GCT   737
Val His Ala Cys Pro Leu Lys Phe Gly Ser Tyr Ala Tyr Thr Thr Ala
            205                 210                 215

GAA GTG GTT TAT TCT TGG ACT CTC GGA AAG AAC AAA TCC GTG GAA GTG   785
Glu Val Val Tyr Ser Trp Thr Leu Gly Lys Asn Lys Ser Val Glu Val
        220                 225                 230

GCA CAG GAT GGT TCT CGC TTG AAC CAG TAT GAC CTT TTG GGC CAT CTT   833
Ala Gln Asp Gly Ser Arg Leu Asn Gln Tyr Asp Leu Leu Gly His Val
    235                 240                 245

GTT GGG ACA GAG ATA ATC CGG TCT AGT ACA GGA GAA TAT GTC GTC ATG   881
Val Gly Thr Glu Ile Ile Arg Ser Ser Thr Gly Glu Tyr Val Val Met
250                 255                 260                 265

ACA ACC CAC TTC CAT CTC AAG CGA AAA ATT GGC TAC TTT GTG ATC CAG   929
Thr Thr His Phe His Leu Lys Arg Lys Ile Gly Tyr Phe Val Ile Gln
                270                 275                 280

ACC TAC TTG CCA TGT ATC ATG ACT GTC ATT CTG TCA CAA GTG TCG TTC   977
Thr Tyr Leu Pro Cys Ile Met Thr Val Ile Leu Ser Gln Val Ser Phe
            285                 290                 295

TGG CTC AAC AGA GAG TCT GTT CCT GCC CGT ACA GTC TTT GGT GTC ACC  1025
Trp Leu Asn Arg Glu Ser Val Pro Ala Arg Thr Val Phe Gly Val Thr
        300                 305                 310
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ACT GTG CTT ACC ATG ACC ACC TTG AGT ATC AGT GCC AGA AAT TCC TTA  1073
Thr Val Leu Thr Met Thr Thr Leu Ser Ile Ser Ala Arg Asn Ser Leu
    315                 320                 325

CCT AAA GTG GCA TAT GCG ACG GCC ATG GAC TGG TTC ATA GCC GTC TGT  1121
Pro Lys Val Ala Tyr Ala Thr Ala Met Asp Trp Phe Ile Ala Val Cys
330                 335                 340                 345

TAT GCC TTT GTA TTT TCT GCA CTG ATT GAA TTT GCC ACT GTC AAC TAT  1169
Tyr Ala Phe Val Phe Ser Ala Leu Ile Glu Phe Ala Thr Val Asn Tyr
                350                 355                 360

TTC ACC AAG CGG AGT TGG GCT TGG GAA GGC AAG AAG GTG CCA GAG GCC  1217
Phe Thr Lys Arg Ser Trp Ala Trp Glu Gly Lys Lys Val Pro Glu Ala
            365                 370                 375

CTG GAG ATG AAG AAG AAA ACA CCA GCA GCC CCA GCA AAG AAA ACC AGC  1265
Leu Glu Met Lys Lys Lys Thr Pro Ala Ala Pro Ala Lys Lys Thr Ser
        380                 385                 390

ACT ACC TTC AAC ATC GTG GGG ACC ACC TAT CCC ATC AAC CTG GCC AAG  1313
Thr Thr Phe Asn Ile Val Gly Thr Thr Tyr Pro Ile Asn Leu Ala Lys
    395                 400                 405

GAC ACT GAA TTT TCC ACC ATC TCC AAG GGC GCT GCT CCC AGT GCC TCC  1361
Asp Thr Glu Phe Ser Thr Ile Ser Lys Gly Ala Ala Pro Ser Ala Ser
410                 415                 420                 425

TCA ACC CCA ACA ATC ATT GCT TCA CCC AAG GCC ACC TAC GTG CAG GAC  1409
Ser Thr Pro Thr Ile Ile Ala Ser Pro Lys Ala Thr Tyr Val Gln Asp
                430                 435                 440

AGC CCG ACT GAG ACC AAG ACC TAC AAC AGT GTC AGC AAG GTT GAC AAA  1457
Ser Pro Thr Glu Thr Lys Thr Tyr Asn Ser Val Ser Lys Val Asp Lys
            445                 450                 455
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ATT TCC CGC ATC ATC TTT CCT GTG CTC TTT GCC ATA TTC AAT CTG GTC 1505
Ile Ser Arg Ile Ile Phe Pro Val Leu Phe Ala Ile Phe Asn Leu Val
460 465 470

TAT TGG GCC ACA TAT GTC AAC CGG GAG TCA GCT ATC AAG GGC ATG ATC 1553
Tyr Trp Ala Thr Tyr Val Asn Arg Glu Ser Ala Ile Lys Gly Met Ile
475 480 485

CGC AAA CAG TAGATAGTGG CAGTGCAGCA ACCAGAGCAC TGTATACCCC 1602
Arg Lys Gln
490

GTGAAGCATC CAGGCACCCA AACCCCGGGG CTCCCC 1638

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 492 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Ile Ile Thr Gln Thr Ser His Cys Tyr Met Thr Ser Leu Gly Ile
1 5 10 15

Leu Phe Leu Ile Asn Ile Leu Pro Gly Thr Thr Gly Gln Gly Glu Ser
20 25 30

Arg Arg Gln Glu Pro Gly Asp Phe Val Lys Gln Asp Ile Gly Gly Leu
35 40 45

Ser Pro Lys His Ala Pro Asp Ile Pro Asp Asp Ser Thr Asp Asn Ile
50 55 60

Thr Ile Phe Thr Arg Ile Leu Asp Arg Leu Leu Asp Gly Tyr Asp Asn
65 70 75 80

Arg Leu Arg Pro Gly Leu Gly Asp Ala Val Thr Glu Val Lys Thr Asp
85 90 95

Ile Tyr Val Thr Ser Phe Gly Pro Val Ser Asp Thr Asp Met Glu Tyr
100 105 110

Thr Ile Asp Val Phe Phe Arg Gln Thr Trp His Asp Glu Arg Leu Lys
115 120 125

Phe Asp Gly Pro Met Lys Ile Leu Pro Leu Asn Asn Leu Leu Ala Ser
130 135 140

Lys Ile Trp Thr Pro Asp Thr Phe Phe His Asn Gly Lys Lys Ser Val
145 150 155 160

Ala His Asn Met Thr Thr Pro Asn Lys Leu Leu Arg Leu Val Asp Asn
165 170 175

Gly Thr Leu Leu Tyr Thr Met Arg Leu Thr Ile His Ala Glu Cys Pro
180 185 190

Met His Leu Glu Asp Phe Pro Met Asp Val His Ala Cys Pro Leu Lys
195 200 205

Phe Gly Ser Tyr Ala Tyr Thr Thr Ala Glu Val Val Tyr Ser Trp Thr
210 215 220

Leu Gly Lys Asn Lys Ser Val Glu Val Ala Gln Asp Gly Ser Arg Leu
225 230 235 240

Asn Gln Tyr Asp Leu Leu Gly His Val Val Gly Thr Glu Ile Ile Arg
245 250 255

Ser Ser Thr Gly Glu Tyr Val Val Met Thr Thr His Phe His Leu Lys
260 265 270

Arg Lys Ile Gly Tyr Phe Val Ile Gln Thr Tyr Leu Pro Cys Ile Met
275 280 285

Thr Val Ile Leu Ser Gln Val Ser Phe Trp Leu Asn Arg Glu Ser Val
290 295 300

Pro Ala Arg Thr Val Phe Gly Val Thr Thr Val Leu Thr Met Thr Thr
305 310 315 320

Leu Ser Ile Ser Ala Arg Asn Ser Leu Pro Lys Val Ala Tyr Ala Thr
325 330 335

Ala Met Asp Trp Phe Ile Ala Val Cys Tyr Ala Phe Val Phe Ser Ala
340 345 350

Leu Ile Glu Phe Ala Thr Val Asn Tyr Phe Thr Lys Arg Ser Trp Ala
355 360 365

Trp Glu Gly Lys Lys Val Pro Glu Ala Leu Glu Met Lys Lys Lys Thr
370 375 380

Pro Ala Ala Pro Ala Lys Lys Thr Ser Thr Thr Phe Asn Ile Val Gly
385 390 395 400

Thr Thr Tyr Pro Ile Asn Leu Ala Lys Asp Thr Glu Phe Ser Thr Ile
405 410 415

Ser Lys Gly Ala Ala Pro Ser Ala Ser Ser Thr Pro Thr Ile Ile Ala
420 425 430

Ser Pro Lys Ala Thr Tyr Val Gln Asp Ser Pro Thr Glu Thr Lys Thr
435 440 445

Tyr Asn Ser Val Ser Lys Val Asp Lys Ile Ser Arg Ile Ile Phe Pro
    450   455   460

Val Leu Phe Ala Ile Phe Asn Leu Val Tyr Trp Ala Thr Tyr Val Asn
465   470   475   480

Arg Glu Ser Ala Ile Lys Gly Met Ile Arg Lys Gln
                485   490

Claims:

1. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_1\beta_3\gamma_2$ subunit combination.

2. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_2\beta_3\gamma_2$ subunit combination.

3. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_5\beta_3\gamma_2$ subunit combination.

4. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_1\beta_1\gamma_{2S}$ subunit combination.

5. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_1\beta_2\gamma_2$ subunit combination.

6. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_3\beta_3\gamma_2$ subunit combination.

7. A stably co-transfected eukaryotic cell line capable of expressing a human $GABA_A$ receptor comprising the $\alpha_6\beta_3\gamma_2$ subunit combination.

8. A membrane preparation containing subunit combinations of the human $GABA_A$ receptor derived from a culture of the stably co-transfected eukaryotic cells as claimed in any one of claims 1 to 3.

9. A membrane preparation containing subunit combinations of the human $GABA_A$ receptor derived from a culture of the stably co-transfected eukaryotic cells as claimed in any one of claims 4 to 7.

10. A preparation as claimed in claim 8 containing a human $GABA_A$ receptor consisting of the $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_3\gamma_{2S}$ or $\alpha_5\beta_3\gamma_{2S}$ subunit combination isolated from stably co-transfected mouse Ltk^- fibroblast cells.

11. A preparation as claimed in claim 9 containing a human $GABA_A$ receptor consisting of the $\alpha_1\beta_1\gamma_{2S}$, $\alpha_1\beta_2\gamma_{2S}$, $\alpha_3\beta_3\gamma_{2S}$ or $\alpha_6\beta_3\gamma_{2S}$ subunit combination isolated from stably co-transfected mouse Ltk^- fibroblast cells.

12. The use of the cell line as claimed in any one of claims 1 to 3, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon the human $GABA_A$ receptor.

13. The use of the cell line as claimed in any one of claims 4 to 7, and membrane preparations derived therefrom, in screening for and designing medicaments which act upon the human $GABA_A$ receptor.

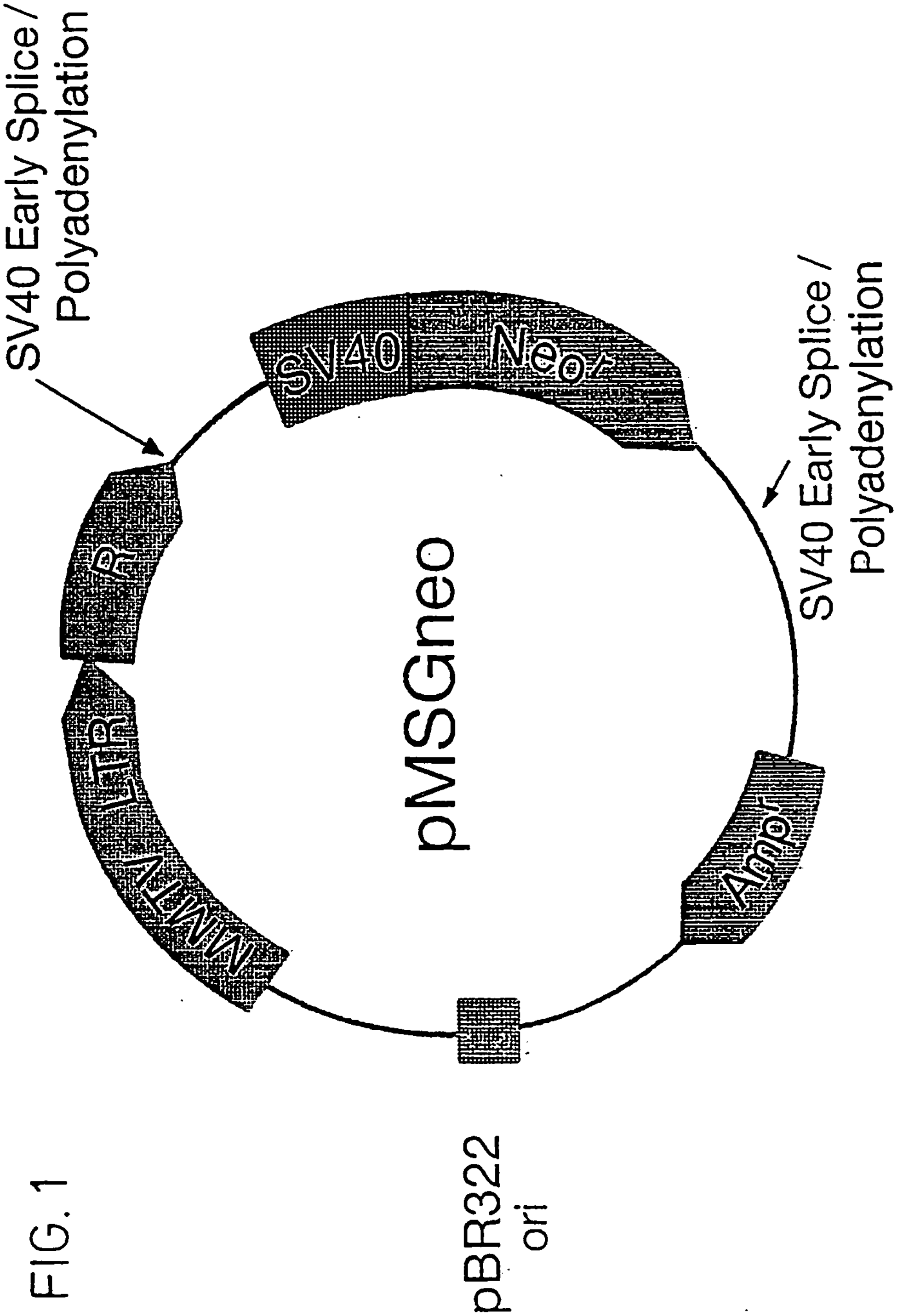
SV40 Early Splice / Polyadenylation
SV40
Neo r
SV40 Early Splice / Polyadenylation
R
pMSGneo
MMTV LTR
Amp r
pBR322 ori

FIG. 1

FIGURE 2

```
        10         20         30         40         50         60         70
CCTAGCGCTC CTCTCCGGCT TCCACCAGCC CATCGCTCCA CGCTCTCTTG GCTGCTGCAG TCTCGGTCTC

        80         90        100        110        120        130        140
TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCCCAA

       150        160        170        180        190        200        210
GTTTCCTATC TCGTCAAGAT CAGGGCAAAA GAAGAAAACA CCGAATTCTG CTTGCCGTTT CAGAGCGGCG

            219         228         237         246         255         264
    >
GTG ATG AAG ACA AAA TTG AAC ATC TAC AAC ATC GAG TTC CTG CTT TTT GTT TTC
    MET Lys Thr Lys Leu Asn Ile Tyr Asn Ile Glu Phe Leu Leu Phe Val Phe

        273         282         291         300         309         318
TTG GTG TGG GAC CCT GCC AGG TTG GTG CTG GCT AAC ATC CAA GAA GAT GAG GCT
Leu Val Trp Asp Pro Ala Arg Leu Val Leu Ala Asn Ile Gln Glu Asp Glu Ala

        327         336         345         354         363         372
AAA AAT AAC ATT ACC ATC TTT ACG AGA ATT CTT GAC AGA CTT CTG GAT GGT TAC
Lys Asn Asn Ile Thr Ile Phe Thr Arg Ile Leu Asp Arg Leu Leu Asp Gly Tyr

        381         390         399         408         417         426
GAT AAT CGG CTT AGA CCA GGA CTG GGA GAC AGT ATT ACT GAA GTC TTC ACT AAC
Asp Asn Arg Leu Arg Pro Gly Leu Gly Asp Ser Ile Thr Glu Val Phe Thr Asn

        435         444         453         462         471         480
ATC TAC GTG ACC AGT TTT GGC CCT GTC TCA GAT ACA GAT ATG GAA TAT ACA ATT
Ile Tyr Val Thr Ser Phe Gly Pro Val Ser Asp Thr Asp MET Glu Tyr Thr Ile

        489         498         507         516         525         534
GAT GTT TTC TTT CGA CAA AAA TGG AAA GAT GAA CGT TTA AAA TTT AAA GGT CCT
Asp Val Phe Phe Arg Gln Lys Trp Lys Asp Glu Arg Leu Lys Phe Lys Gly Pro

        543         552         561         570         579         588
ATG AAT ATC CTT CGA CTA AAC AAT TTA ATG GCT AGC AAA ATC TGG ACT CCA GAT
MET Asn Ile Leu Arg Leu Asn Asn Leu MET Ala Ser Lys Ile Trp Thr Pro Asp

        597         606         615         624         633         642
ACC TTT TTT CAC AAT GGG AAG AAA TCA GTA GCT CAT AAT ATG ACA ATG CCA AAT
Thr Phe Phe His Asn Gly Lys Lys Ser Val Ala His Asn MET Thr MET Pro Asn

        651         660         669         678         687         696
AAG TTG CTT CGA ATT CAG GAT GAT GGG ACT CTG CTG TAT ACC ATG AGG CTT ACA
Lys Leu Leu Arg Ile Gln Asp Asp Gly Thr Leu Leu Tyr Thr MET Arg Leu Thr

        705         714         723         732         741         750
GTT CAA GCT GAA TGC CCA ATG CAC TTG GAG GAT TTC CCA ATG GAT GCT CAT TCA
Val Gln Ala Glu Cys Pro MET His Leu Glu Asp Phe Pro MET Asp Ala His Ser
```

FIGURE 2 (CONTINUED)

		759			768			777			786			795			804
TGT	CCT	CTG	AAA	TTT	GGC	AGC	TAT	GCA	TAT	ACA	ACT	TCA	GAG	GTC	ACT	TAT	ATT
Cys	Pro	Leu	Lys	Phe	Gly	Ser	Tyr	Ala	Tyr	Thr	Thr	Ser	Glu	Val	Thr	Tyr	Ile
		813			822			831			840			849			858
TGG	ACT	TAC	AAT	GCA	TCT	GAT	TCA	GTA	CAG	GTT	GCT	CCT	GAT	GGC	TCT	AGG	TTA
Trp	Thr	Tyr	Asn	Ala	Ser	Asp	Ser	Val	Gln	Val	Ala	Pro	Asp	Gly	Ser	Arg	Leu
		867			876			885			894			903			912
AAT	CAA	TAT	GAC	CTG	CTG	GGC	CAA	TCA	ATC	GGA	AAG	GAG	ACA	ATT	AAA	TCC	AGT
Asn	Gln	Tyr	Asp	Leu	Leu	Gly	Gln	Ser	Ile	Gly	Lys	Glu	Thr	Ile	Lys	Ser	Ser
		921			930			939			948			957			966
ACA	GGT	GAA	TAT	ACT	GTA	ATG	ACA	GCT	CAT	TTC	CAC	CTG	AAA	AGA	AAA	ATT	GGG
Thr	Gly	Glu	Tyr	Thr	Val	MET	Thr	Ala	His	Phe	His	Leu	Lys	Arg	Lys	Ile	Gly
		975			984			993			1002			1011			1020
TAT	TTT	GTG	ATT	CAA	ACC	TAT	CTG	CCT	TGC	ATC	ATG	ACT	GTC	ATT	CTC	TCC	CAA
Tyr	Phe	Val	Ile	Gln	Thr	Tyr	Leu	Pro	Cys	Ile	MET	Thr	Val	Ile	Leu	Ser	Gln
		1029			1038			1047			1056			1065			1074
GTT	TCA	TTC	TGG	CTT	AAC	AGA	GAA	TCT	GTG	CCT	GCA	AGA	ACT	GTG	TTT	GGA	GTA
Val	Ser	Phe	Trp	Leu	Asn	Arg	Glu	Ser	Val	Pro	Ala	Arg	Thr	Val	Phe	Gly	Val
		1083			1092			1101			1110			1119			1128
ACA	ACT	GTC	CTA	ACA	ATG	ACA	ACT	CTA	AGC	ATC	AGT	GCT	CGG	AAT	TCT	CTC	CCC
Thr	Thr	Val	Leu	Thr	MET	Thr	Thr	Leu	Ser	Ile	Ser	Ala	Arg	Asn	Ser	Leu	Pro
		1137			1146			1155			1164			1173			1182
AAA	GTG	GCT	TAT	GCA	ACT	GCC	ATG	GAC	TGG	TTT	ATT	GCT	GTT	TGT	TAT	GCA	TTT
Lys	Val	Ala	Tyr	Ala	Thr	Ala	MET	Asp	Trp	Phe	Ile	Ala	Val	Cys	Tyr	Ala	Phe
		1191			1200			1209			1218			1227			1236
GTG	TTC	TCT	GCC	CTA	ATT	GAA	TTT	GCA	ACT	GTT	AAT	TAC	TTC	ACC	AAA	AGA	GGA
Val	Phe	Ser	Ala	Leu	Ile	Glu	Phe	Ala	Thr	Val	Asn	Tyr	Phe	Thr	Lys	Arg	Gly
		1245			1254			1263			1272			1281			1290
TGG	ACT	TGG	GAT	GGG	AAG	AGT	GTA	GTA	AAT	GAC	AAG	AAA	AAA	GAA	AAG	GCT	TCC
Trp	Thr	Trp	Asp	Gly	Lys	Ser	Val	Val	Asn	Asp	Lys	Lys	Lys	Glu	Lys	Ala	Ser
		1299			1308			1317			1326			1335			1344
GTT	ATG	ATA	CAG	AAC	AAC	GCT	TAT	GCA	GTG	GCT	GTT	GCC	AAT	TAT	GCC	CCG	AAT
Val	MET	Ile	Gln	Asn	Asn	Ala	Tyr	Ala	Val	Ala	Val	Ala	Asn	Tyr	Ala	Pro	Asn
		1353			1362			1371			1380			1389			1398
CTT	TCA	AAA	GAT	CCA	GTT	CTC	TCC	ACC	ATC	TCC	AAG	AGT	GCA	ACC	ACG	CCA	GAA
Leu	Ser	Lys	Asp	Pro	Val	Leu	Ser	Thr	Ile	Ser	Lys	Ser	Ala	Thr	Thr	Pro	Glu
		1407			1416			1425			1434			1443			1452
CCC	AAC	AAG	AAG	CCA	GAA	AAC	AAG	CCA	GCT	GAA	GCA	AAG	AAA	ACT	TTC	AAC	AGT
Pro	Asn	Lys	Lys	Pro	Glu	Asn	Lys	Pro	Ala	Glu	Ala	Lys	Lys	Thr	Phe	Asn	Ser

FIGURE 2 (CONTINUED)

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       1461          1470          1479          1488          1497          1506
GTT AGC AAA ATT GAC AGA ATG TCC AGA ATA GTT TTT CCA GTT TTG TTT GGT ACC
Val Ser Lys Ile Asp Arg MET Ser Arg Ile Val Phe Pro Val Leu Phe Gly Thr

       1515          1524          1533          1542          1551          1560
TTT AAT TTA GTT TAC TGG GCT ACA TAT TTA AAC AGA GAA CCT GTA TTA GGG GTC
Phe Asn Leu Val Tyr Trp Ala Thr Tyr Leu Asn Arg Glu Pro Val Leu Gly Val

       1569        1579        1589        1599        1609        1619        1629
          >
AGT CCT TGA ATTGAGACCC ATGTTATCTT TGGGATGTAT AGCAACATTA AATTTGGTTT GTTTTGCTAT
Ser Pro

      1639       1649       1659       1669       1679       1689       1699
GTACAGTCTG ACTAATAACT GCTAATTTGT GATCCAACAT GTACAGTATG TATATAGTGA CATAGCTTAC

      1709       1719       1729       1739       1749       1759       1769
CAGTAGACCT TTAATGGAGA CATGCATTTG CTAACTCATG GAACTGCAGA CAGAAAGCAC TCCATGCGAA

      1779       1789       1799       1809       1819       1829       1839
AACAGCCATT GCCTTTTTTA AAGATTTACC CTAGGACCTG ATTTAAAGTG AATTTCAAGT GACCTGATTA

      1849       1859       1869       1879       1889       1899       1909
ATTTCCTATT CTTCCAAATG AGATGAAAAT GGGGATCCTG TACAACCCTT TGTGGACCCT TTTGGTTTAG

      1919       1929       1939       1949       1959       1969       1979
CTCTTAAGTA GGGGTATTTT CTACTGTTGC TTAATTATGA TGGAAGATAA CATTGTCATT CCTAGATGAA

      1989       1999       2009       2019       2029       2039       2049
TCCTTTGAAG TAACAAACAT TGTATCTGAC ATCAGCTCTG TTCATGAGTG CTCAGAGTCC CTGCTAATGT

      2059       2069       2079       2089       2099       2109       2119
AATTGGAAGC TTGGTACACA TAAGAAAAAC TAGAGATTTG AAATCTAGCT ATGAATTACT CTATATAGTA

      2129       2139       2149       2159       2169       2179       2189
TCTATAGCCA TGTACATATT ACAGCATGAC AAGCTCGAAA TAATTATGAG TCAGCCCGAA AGATGTTAAT
```

FIGURE 3

```
        10         20         30         40         50         60         70
GAATTCCCTT GTTTCAGTTC ATTCATCCTT CTCTCCTTTC CGCTCAGACT GTAGAGCTCG GTCTCTCCAA

        80         89          98         107         116         125
                   >
GTTTGTGCCT AAGAAG ATG ATA ATC ACA CAA ACA AGT CAC TGT TAC ATG ACC AGC
                  MET Ile Ile Thr Gln Thr Ser His Cys Tyr MET Thr Ser

         134         143         152         161         170         179
CTT GGG ATT CTT TTC CTG ATT AAT ATT CTC CCT GGA ACC ACT GGT CAA GGG GAA
Leu Gly Ile Leu Phe Leu Ile Asn Ile Leu Pro Gly Thr Thr Gly Gln Gly Glu

         188         197         206         215         224         233
TCA AGA CGA CAA GAA CCC GGG GAC TTT GTG AAG CAG GAC ATT GGC GGG CTG TCT
Ser Arg Arg Gln Glu Pro Gly Asp Phe Val Lys Gln Asp Ile Gly Gly Leu Ser

         242         251         260         269         278         287
CCT AAG CAT GCC CCA GAT ATT CCT GAT GAC AGC ACT GAC AAC ATC ACT ATC TTC
Pro Lys His Ala Pro Asp Ile Pro Asp Asp Ser Thr Asp Asn Ile Thr Ile Phe

         296         305         314         323         332         341
ACC AGA ATC TTG GAT CGT CTT CTG GAC GGC TAT GAC AAC CGG CTG CGA CCT GGG
Thr Arg Ile Leu Asp Arg Leu Leu Asp Gly Tyr Asp Asn Arg Leu Arg Pro Gly

         350         359         368         377         386         395
CTT GGA GAT GCA GTG ACT GAA GTG AAG ACT GAC ATC TAC GTG ACC AGT TTT GGC
Leu Gly Asp Ala Val Thr Glu Val Lys Thr Asp Ile Tyr Val Thr Ser Phe Gly

         404         413         422         431         440         449
CCT GTG TCA GAC ACT GAC ATG GAG TAC ACT ATT GAT GTA TTT TTT CGG CAG ACA
Pro Val Ser Asp Thr Asp MET Glu Tyr Thr Ile Asp Val Phe Phe Arg Gln Thr

         458         467         476         485         494         503
TGG CAT GAT GAA AGA CTG AAA TTT GAT GGC CCC ATG AAG ATC CTT CCA CTG AAC
Trp His Asp Glu Arg Leu Lys Phe Asp Gly Pro MET Lys Ile Leu Pro Leu Asn

         512         521         530         539         548         557
AAT CTC CTG GCT AGT AAG ATC TGG ACA CCG GAC ACC TTC TTC CAC AAT GGC AAG
Asn Leu Leu Ala Ser Lys Ile Trp Thr Pro Asp Thr Phe Phe His Asn Gly Lys

         566         575         584         593         602         611
AAA TCA GTG GCT CAT AAC ATG ACC ACG CCC AAC AAG CTG CTC AGA TTG GTG GAC
Lys Ser Val Ala His Asn MET Thr Thr Pro Asn Lys Leu Leu Arg Leu Val Asp

         620         629         638         647         656         665
AAC GGA ACC CTC CTC TAT ACA ATG AGG TTA ACA ATT CAT GCT GAG TGT CCC ATG
Asn Gly Thr Leu Leu Tyr Thr MET Arg Leu Thr Ile His Ala Glu Cys Pro MET

         674         683         692         701         710         719
CAT TTG GAA GAT TTT CCC ATG GAT GTG CAT GCC TGC CCA CTG AAG TTT GGA AGC
His Leu Glu Asp Phe Pro MET Asp Val His Ala Cys Pro Leu Lys Phe Gly Ser
```

FIGURE 3 (CONTINUED)

```
        728         737         746         755         764         773
TAT GCC TAT ACA ACA GCT GAA GTG GTT TAT TCT TGG ACT CTC GGA AAG AAC AAA
Tyr Ala Tyr Thr Thr Ala Glu Val Val Tyr Ser Trp Thr Leu Gly Lys Asn Lys

        782         791         800         809         818         827
TCC GTG GAA GTG GCA CAG GAT GGT TCT CGC TTG AAC CAG TAT GAC CTT TTG GGC
Ser Val Glu Val Ala Gln Asp Gly Ser Arg Leu Asn Gln Tyr Asp Leu Leu Gly

        836         845         854         863         872         881
CAT GTT GTT GGG ACA GAG ATA ATC CGG TCT AGT ACA GGA GAA TAT GTC GTC ATG
His Val Val Gly Thr Glu Ile Ile Arg Ser Ser Thr Gly Glu Tyr Val Val MET

        890         899         908         917         926         935
ACA ACC CAC TTC CAT CTC AAG CGA AAA ATT GGC TAC TTT GTG ATC CAG ACC TAC
Thr Thr His Phe His Leu Lys Arg Lys Ile Gly Tyr Phe Val Ile Gln Thr Tyr

        944         953         962         971         980         989
TTG CCA TGT ATC ATG ACT GTC ATT CTG TCA CAA GTG TCG TTC TGG CTC AAC AGA
Leu Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser Phe Trp Leu Asn Arg

        998        1007        1016        1025        1034        1043
GAG TCT GTT CCT GCC CGT ACA GTC TTT GGT GTC ACC ACT GTG CTT ACC ATG ACC
Glu Ser Val Pro Ala Arg Thr Val Phe Gly Val Thr Thr Val Leu Thr MET Thr

       1052        1061        1070        1079        1088        1097
ACC TTG AGT ATC AGT GCC AGA AAT TCC TTA CCT AAA GTG GCA TAT GCG ACG GCC
Thr Leu Ser Ile Ser Ala Arg Asn Ser Leu Pro Lys Val Ala Tyr Ala Thr Ala

       1106        1115        1124        1133        1142        1151
ATG GAC TGG TTC ATA GCC GTC TGT TAT GCC TTT GTA TTT TCT GCA CTG ATT GAA
MET Asp Trp Phe Ile Ala Val Cys Tyr Ala Phe Val Phe Ser Ala Leu Ile Glu

       1160        1169        1178        1187        1196        1205
TTT GCC ACT GTC AAC TAT TTC ACC AAG CGG AGT TGG GCT TGG GAA GGC AAG AAG
Phe Ala Thr Val Asn Tyr Phe Thr Lys Arg Ser Trp Ala Trp Glu Gly Lys Lys

       1214        1223        1232        1241        1250        1259
GTG CCA GAG GCC CTG GAG ATG AAG AAG AAA ACA CCA GCA GCC CCA GCA AAG AAA
Val Pro Glu Ala Leu Glu MET Lys Lys Lys Thr Pro Ala Ala Pro Ala Lys Lys

       1268        1277        1286        1295        1304        1313
ACC AGC ACT ACC TTC AAC ATC GTG GGG ACC ACC TAT CCC ATC AAC CTG GCC AAG
Thr Ser Thr Thr Phe Asn Ile Val Gly Thr Thr Tyr Pro Ile Asn Leu Ala Lys

       1322        1331        1340        1349        1358        1367
GAC ACT GAA TTT TCC ACC ATC TCC AAG GGC GCT GCT CCC AGT GCC TCC TCA ACC
Asp Thr Glu Phe Ser Thr Ile Ser Lys Gly Ala Ala Pro Ser Ala Ser Ser Thr

       1376        1385        1394        1403        1412        1421
CCA ACA ATC ATT GCT TCA CCC AAG GCC ACC TAC GTG CAG GAC AGC CCG ACT GAG
Pro Thr Ile Ile Ala Ser Pro Lys Ala Thr Tyr Val Gln Asp Ser Pro Thr Glu
```

FIGURE 3 (CONTINUED)

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     1430        1439        1448        1457        1466        1475

ACC AAG ACC TAC AAC AGT GTC AGC AAG GTT GAC AAA ATT TCC CGC ATC ATC TTT
Thr Lys Thr Tyr Asn Ser Val Ser Lys Val Asp Lys Ile Ser Arg Ile Ile Phe

     1484        1493        1502        1511        1520        1529

CCT GTG CTC TTT GCC ATA TTC AAT CTG GTC TAT TGG GCC ACA TAT GTC AAC CGG
Pro Val Leu Phe Ala Ile Phe Asn Leu Val Tyr Trp Ala Thr Tyr Val Asn Arg

     1538        1547        1556        1565       1575       1585
                                            >
GAG TCA GCT ATC AAG GGC ATG ATC CGC AAA CAG TAG ATAGTGGCAG TGCAGCAACC
Glu Ser Ala Ile Lys Gly MET Ile Arg Lys Gln

     1595       1605       1615       1625       1635

AGAGCACTGT ATACCCCGTG AAGCATCCAG GCACCCAAAC CCCGGGGCTC CCC
```

FIGURE 4

```
        10         20         30         40         50         60         70
GAATTCCCCC CTTGCAGGCC GAGCCGGGGC CCTGCGCCCT CCCCCTCCGC CCAGCTCGGC CAAGGGCGCA

        80         90        100        110        120        130        140
TTTGCTGAGC GTCTGGCGGC CTCTACCGGA GCACCTCTGC AGAGGGCCGA TCCTCCAGCC CAGAGACGAC

       150        160        170        180        190        200        210
ATGTGGCGCT CGGGCGAGTG CCTTGCAGAG AGAGGAGTAG CTTGCTGGCT TTGAACGCGT GGCGTGGCAG

       220        230        240        250        260        270        280
ATATTTCAGA AAGCTTCAAG AACAAGCTGG AGAAGGGAAG AGTTATTCCT CCATATTCAC CTGCTTCAAC

       290        300         309         318         327         336
                  >
TACTATTCTT ATTGGGA ATG GAC AAT GGA ATG TTC TCT GGT TTT ATC ATG ATC AAA
                   MET Asp Asn Gly MET Phe Ser Gly Phe Ile MET Ile Lys

        345         354         363         372         381         390
AAC CTC CTT CTC TTT TGT ATT TCC ATG AAC TTA TCC AGT CAC TTT GGC TTT TCA
Asn Leu Leu Leu Phe Cys Ile Ser MET Asn Leu Ser Ser His Phe Gly Phe Ser

        399         408         417         426         435         444
CAG ATG CCA ACC AGT TCA GTG AAA GAT GAG ACC AAT GAC AAC ATC ACG ATA TTT
Gln MET Pro Thr Ser Ser Val Lys Asp Glu Thr Asn Asp Asn Ile Thr Ile Phe

        453         462         471         480         489         498
ACC AGG ATC TTG GAT GGG CTC TTG GAT GGC TAC GAC AAC AGA CTT CGG CCC GGG
Thr Arg Ile Leu Asp Gly Leu Leu Asp Gly Tyr Asp Asn Arg Leu Arg Pro Gly

        507         516         525         534         543         552
CTG GGA GAG CGC ATC ACT CAG GTG AGG ACC GAC ATC TAC GTC ACC AGC TTC GGC
Leu Gly Glu Arg Ile Thr Gln Val Arg Thr Asp Ile Tyr Val Thr Ser Phe Gly

        561         570         579         588         597         606
CCG GTG TCC GAC ACG GAA ATG GAG TAC ACC ATA GAC GTG TTT TTC CGA CAA AGC
Pro Val Ser Asp Thr Glu MET Glu Tyr Thr Ile Asp Val Phe Phe Arg Gln Ser

        615         624         633         642         651         660
TGG AAA GAT GAA AGG CTT CGG TTT AAG GGG CCC ATG CAG CGC CTC CCT CTC AAC
Trp Lys Asp Glu Arg Leu Arg Phe Lys Gly Pro MET Gln Arg Leu Pro Leu Asn

        669         678         687         696         705         714
AAC CTC CTT GCC AGC AAG ATC TGG ACC CCA GAC ACG TTC TTC CAC AAC GGG AAG
Asn Leu Leu Ala Ser Lys Ile Trp Thr Pro Asp Thr Phe Phe His Asn Gly Lys

        723         732         741         750         759         768
AAG TCC ATC GCT CAC AAC ATG ACC ACG CCC AAC AAG CTG CTG CGG CTG GAG GAC
Lys Ser Ile Ala His Asn MET Thr Thr Pro Asn Lys Leu Leu Arg Leu Glu Asp
```

FIGURE 4 (CONTINUED)

```
        777         786         795         804         813         822
GAC GGC ACC CTG CTC TAC ACC ATG CGC TTG ACC ATC TCT GCA GAG TGC CCC ATG
Asp Gly Thr Leu Leu Tyr Thr MET Arg Leu Thr Ile Ser Ala Glu Cys Pro MET

        831         840         849         858         867         876
CAG CTT GAG GAC TTC CCG ATG GAT GCG CAC GCT TGC CCT CTG AAA TTT GGC AGC
Gln Leu Glu Asp Phe Pro MET Asp Ala His Ala Cys Pro Leu Lys Phe Gly Ser

        885         894         903         912         921         930
TAT GCG TAC CCT AAT TCT GAA GTC GTT TAC GTC TGG ACC AAC GGC TCC ACC AAG
Tyr Ala Tyr Pro Asn Ser Glu Val Val Tyr Val Trp Thr Asn Gly Ser Thr Lys

        939         948         957         966         975         984
TCG GTG GTG GTG GCG GAA GAT GGC TCC AGA CTG AAC CAG TAC CAC CTG ATG GGG
Ser Val Val Val Ala Glu Asp Gly Ser Arg Leu Asn Gln Tyr His Leu MET Gly

        993        1002        1011        1020        1029        1038
CAG ACG GTG GGC ACT GAG AAC ATC AGC ACC AGC ACA GGC GAA TAC ACA ATC ATG
Gln Thr Val Gly Thr Glu Asn Ile Ser Thr Ser Thr Gly Glu Tyr Thr Ile MET

       1047        1056        1065        1074        1083        1092
ACA GCT CAC TTC CAC CTG AAA AGG AAG ATT GGC TAC TTT GTC ATC CAG ACC TAC
Thr Ala His Phe His Leu Lys Arg Lys Ile Gly Tyr Phe Val Ile Gln Thr Tyr

       1101        1110        1119        1128        1137        1146
CTT CCC TGC ATA ATG ACC GTG ATC TTA TCA CAG GTG TCC TTT TGG CTG AAC CGG
Leu Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser Phe Trp Leu Asn Arg

       1155        1164        1173        1182        1191        1200
GAA TCA GTC CCA GCC AGG ACA GTT TTT GGG GTC ACC ACG GTG CTG ACC ATG ACG
Glu Ser Val Pro Ala Arg Thr Val Phe Gly Val Thr Thr Val Leu Thr MET Thr

       1209        1218        1227        1236        1245        1254
ACC CTC AGC ATC AGC GCC AGG AAC TCT CTG CCC AAA GTG GCC TAC GCC ACC GCC
Thr Leu Ser Ile Ser Ala Arg Asn Ser Leu Pro Lys Val Ala Tyr Ala Thr Ala

       1263        1272        1281        1290        1299        1308
ATG GAC TGG TTC ATA GCT GTG TGC TAT GCC TTC GTC TTC TCG GCG CTG ATA GAG
MET Asp Trp Phe Ile Ala Val Cys Tyr Ala Phe Val Phe Ser Ala Leu Ile Glu

       1317        1326        1335        1344        1353        1362
TTT GCC ACG GTC AAT TAC TTT ACC AAG AGA GGC TGG GCC TGG GAT GGC AAA AAA
Phe Ala Thr Val Asn Tyr Phe Thr Lys Arg Gly Trp Ala Trp Asp Gly Lys Lys

       1371        1380        1389        1398        1407        1416
GCC TTG GAA GCA GCC AAG ATC AAG AAA AAG CGT GAA GTC ATA CTA AAT AAG TCA
Ala Leu Glu Ala Ala Lys Ile Lys Lys Lys Arg Glu Val Ile Leu Asn Lys Ser

       1425        1434        1443        1452        1461        1470
ACA AAC GCT TTT ACA ACT GGG AAG ATG TCT CAC CCC CCA AAC ATT CCG AAG GAA
Thr Asn Ala Phe Thr Thr Gly Lys MET Ser His Pro Pro Asn Ile Pro Lys Glu
```

FIGURE 4 (CONTINUED)

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     1479         1488         1497         1506         1515         1524
CAG ACC CCA GCA GGG ACG TCG AAT ACA ACC TCA GTC TCA GTA AAA CCC TCT GAA
Gln Thr Pro Ala Gly Thr Ser Asn Thr Thr Ser Val Ser Val Lys Pro Ser Glu

     1533         1542         1551         1560         1569         1578
GAG AAG ACT TCT GAA AGC AAA AAG ACT TAC AAC AGT ATC AGC AAA ATT GAC AAA
Glu Lys Thr Ser Glu Ser Lys Lys Thr Tyr Asn Ser Ile Ser Lys Ile Asp Lys

     1587         1596         1605         1614         1623         1632
ATG TCC CGA ATC GTA TTC CCA GTC TTG TTC GGC ACT TTC AAC TTA GTT TAC TGG
MET Ser Arg Ile Val Phe Pro Val Leu Phe Gly Thr Phe Asn Leu Val Tyr Trp

     1641         1650         1659         1668         1677         1686
                                                                        >
GCA ACG TAT TTG AAT AGG GAG CCG GTG ATA AAA GGA GCC GCC TCT CCA AAA TAA
Ala Thr Tyr Leu Asn Arg Glu Pro Val Ile Lys Gly Ala Ala Ser Pro Lys  .

      1696       1706       1716       1726       1736       1746       1756
CCGGCCACAC TCCCAAACTC CAAGACAGCC ATACTTCCAG CGAAATGGTA CCAAGGAGAG GTTTTGCTCA

      1766       1776       1786       1796       1806       1816       1826
CAGGGACTCT CCATATGTGA GCACTATCTT TCAGGAAATT TTTGCATGTT TAATAATATG TACAAATAAT

      1836       1846       1856       1866       1876       1886       1896
ATTGCCTTGA TGTTTCTATA TGTAACTTCA GATGTTTCCA AGATGTCCCA TTGATAATTC GAGCAAACAA

      1906       1916       1926       1936       1946       1956       1966
CTTTCTGGAA AAACAGGATA CGATGACTGA CACTCAGATG CCCAGTATCA TACGTTGATA GTTTACAAAC

      1976       1986       1996       2006       2016       2026       2036
AAGATACGTA TATTTTTAAC TGCTTCAAGT GTTACCTAAC AATGTTTTTT ATACTTCAAA TGTCATTTCA

      2046       2056       2066       2076       2086       2096       2106
TACAAATTTT CCCAGTGAAT AAATATTTTA GGAAACTCTC CATGATTATT AGAAGACCAA CTATATTGCG

      2116       2126       2136       2146       2156       2166       2176
AGAAACAGAG ATCATAAAGA GCACGTTTTC CATTATGAGG AAACTTGGAC ATTTATGTAC AAAATGAATT

      2186       2196       2206       2216       2226       2236       2246
GCCTTTGATA ATTCTTACTG TTCTGAAATT AGGAAAGTAC TTGCATGATC TTACACGAAG AAATAGAATA

      2256       2266       2276       2286       2296       2306
GGCAAACTTT TATGTAGGCA GATTAATAAC AGAAATACAT CATATGTTAG ATACACAAAA TATT
```

FIGURE 5

```
          10         20         29       38       47       56
                                >
AATTCTGCAT TTCAGTGCAC TGCAGG ATG GCG TCA TCT CTG CCC TGG CTG TGC ATT
                             MET Ala Ser Ser Leu Pro Trp Leu Cys Ile

        65          74          83          92          101         110
ATT CTG TGG CTA GAA AAT GCC CTA GGG AAA CTC GAA GTT GAA GGC AAC TTC TAC
Ile Leu Trp Leu Glu Asn Ala Leu Gly Lys Leu Glu Val Glu Gly Asn Phe Tyr

        119         128         137         146         155         164
TCA GAA AAC GTC AGT CGG ATC CTG GAC AAC TTG CTT GAA GGC TAT GAC AAT CGG
Ser Glu Asn Val Ser Arg Ile Leu Asp Asn Leu Leu Glu Gly Tyr Asp Asn Arg

        173         182         191         200         209         218
CTG CGG CCG GGA TTT GGA GGT GCT GTC ACT GAA GTC AAA ACA GAC ATT TAT GTG
Leu Arg Pro Gly Phe Gly Gly Ala Val Thr Glu Val Lys Thr Asp Ile Tyr Val

        227         236         245         254         263         272
ACC AGT TTT GGG CCC GTG TCA GAT GTG GAG ATG GAG TAT ACG ATG GAT GTT TTT
Thr Ser Phe Gly Pro Val Ser Asp Val Glu MET Glu Tyr Thr MET Asp Val Phe

        281         290         299         308         317         326
TTT CGC CAG ACC TGG ACT GAT GAG AGG TTG AAG TTT GGG GGG CCA ACT GAG ATT
Phe Arg Gln Thr Trp Thr Asp Glu Arg Leu Lys Phe Gly Gly Pro Thr Glu Ile

        335         344         353         362         371         380
CTG AGT CTG AAT AAT TTG ATG GTC AGT AAA ATC TGG ACG CCT GAC ACC TTT TTC
Leu Ser Leu Asn Asn Leu MET Val Ser Lys Ile Trp Thr Pro Asp Thr Phe Phe

        389         398         407         416         425         434
AGA AAT GGT AAA AAG TCC ATT GCT CAC AAC ATG ACA ACT CCT AAT AAA CTC TTC
Arg Asn Gly Lys Lys Ser Ile Ala His Asn MET Thr Thr Pro Asn Lys Leu Phe

        443         452         461         470         479         488
AGA ATA ATG CAG AAT GGA ACC ATT TTA TAC ACC ATG AGG CTT ACC ATC AAT GCT
Arg Ile MET Gln Asn Gly Thr Ile Leu Tyr Thr MET Arg Leu Thr Ile Asn Ala

        497         506         515         524         533         542
GAC TGT CCC ATG AGG CTG GTT AAC TTT CCT ATG GAT GGG CAT GCT TGT CCA CTC
Asp Cys Pro MET Arg Leu Val Asn Phe Pro MET Asp Gly His Ala Cys Pro Leu

        551         560         569         578         587         596
AAG TTT GGG AGC TAT GCT TAT CCC AAA AGT GAA ATC ATA TAT ACG TGG AAA AAA
Lys Phe Gly Ser Tyr Ala Tyr Pro Lys Ser Glu Ile Ile Tyr Thr Trp Lys Lys

        605         614         623         632         641         650
GGA CCA CTT TAC TCA GTA GAA GTC CCA GAA GAA TCT TCA AGC CTT CTC CAG TAT
Gly Pro Leu Tyr Ser Val Glu Val Pro Glu Glu Ser Ser Ser Leu Leu Gln Tyr

        659         668         677         686         695         704
GAT CTG ATT GGA CAA ACA GTA TCT AGT GAG ACA ATT AAA TCT AAC ACA GGT GAA
Asp Leu Ile Gly Gln Thr Val Ser Ser Glu Thr Ile Lys Ser Asn Thr Gly Glu
```

FIGURE 5 (CONTINUED)

713 722 731 740 749 758

TAC GTT ATA ATG ACA GTT TAC TTC CAC TTG CAA AGG AAG ATG GGC TAC TTC ATG

Tyr Val Ile MET Thr Val Tyr Phe His Leu Gln Arg Lys MET Gly Tyr Phe MET

767 776 785 794 803 812

ATA CAG ATA TAC ACT CCT TGC ATT ATG ACA GTC ATT CTT TCC CAG GTG TCT TTC

Ile Gln Ile Tyr Thr Pro Cys Ile MET Thr Val Ile Leu Ser Gln Val Ser Phe

821 830 839 848 857 866

TGG ATT AAT AAG GAG TCC GTC CCA GCA AGA ACT GTT CTT GGG ATC ACC ACT GTT

Trp Ile Asn Lys Glu Ser Val Pro Ala Arg Thr Val Leu Gly Ile Thr Thr Val

875 884 893 902 911 920

TTA ACT ATG ACC ACT TTG AGC ATC AGT GCC CGG CAC TCT TTG CCA AAA GTG TCA

Leu Thr MET Thr Thr Leu Ser Ile Ser Ala Arg His Ser Leu Pro Lys Val Ser

929 938 947 956 965 974

TAT GCC ACT GCC ATG GAT TGG TTC ATA GCT GTT TGC TTT GCA TTC GTC TTC TCT

Tyr Ala Thr Ala MET Asp Trp Phe Ile Ala Val Cys Phe Ala Phe Val Phe Ser

983 992 1001 1010 1019 1028

GCT CTT ATC GAG TTC GCA GCT GTC AAC TAC TTT ACC AAT CTT CAG ACA CAG AAG

Ala Leu Ile Glu Phe Ala Ala Val Asn Tyr Phe Thr Asn Leu Gln Thr Gln Lys

1037 1046 1055 1064 1073 1082

GCG AAA AGG AAG GCA CAG TTT GCA GCC CCA CCC ACA GTG ACA ATA TCA AAA GCT

Ala Lys Arg Lys Ala Gln Phe Ala Ala Pro Pro Thr Val Thr Ile Ser Lys Ala

1091 1100 1109 1118 1127 1136

ACT GAA CCT TTG GAA GCT GAG ATT GTT TTG CAT CCT GAC TCC AAA TAT CAT CTG

Thr Glu Pro Leu Glu Ala Glu Ile Val Leu His Pro Asp Ser Lys Tyr His Leu

1145 1154 1163 1172 1181 1190

AAG AAA AGG ATC ACT TCT CTG TCT TTG CCA ATA GTT TCA TCT TCC GAG GCC AAT

Lys Lys Arg Ile Thr Ser Leu Ser Leu Pro Ile Val Ser Ser Ser Glu Ala Asn

1199 1208 1217 1226 1235 1244

AAA GTG CTC ACG AGA GCG CCC ATC TTA CAA TCA ACA CCT GTC ACA CCC CCA CCA

Lys Val Leu Thr Arg Ala Pro Ile Leu Gln Ser Thr Pro Val Thr Pro Pro Pro

1253 1262 1271 1280 1289 1298

CTC CCG CCA GCC TTT GGA GGC ACC AGT AAA ATA GAC CAG TAT TCT CGA ATT CTC

Leu Pro Pro Ala Phe Gly Gly Thr Ser Lys Ile Asp Gln Tyr Ser Arg Ile Leu

1307 1316 1325 1334 1343 1352

TTC CCA GTT GCA TTT GCA GGA TTC AAC CTT GTG TAC TGG GTA GTT TAT CTT TCC

Phe Pro Val Ala Phe Ala Gly Phe Asn Leu Val Tyr Trp Val Val Tyr Leu Ser

1361 1370 1379 1388 1398 1408

>

AAA GAT ACA ATG GAA GTG AGT AGC AGT GTT GAA TAG CTTTTCCAGG ACAACCTGAA

Lys Asp Thr MET Glu Val Ser Ser Ser Val Glu .

FIGURE 6

```
        10         20         30         40         50         60         70
GAATTCCGCG CGGGGAAGGG AAGAAGAGGA CGAGGTGGCG CAGAGACCGC GGGAGAACAC AGTGCCTCCG

        80         90        100        110        120        130        140
GAGGAAATCT GCTCGGTCCC CGGCAGCCGC GCTTCCCCTT TGATGTTTTG GTACGCCGTG GCCATGCGCC

       150        160        170        180        190        200        210
TCACATTAGA ATTACTGCAC TGGGCAGACT AAGTTGGATC TCCTCTCTTC AGTGAAACCC TCAATTCCAT

       220           230         239         248         257         266
               >
CAAAAACTAA AGGG ATG TGG AGA GTG CGG AAA AGG GGC TAC TTT GGG ATT TGG TCC
                MET Trp Arg Val Arg Lys Arg Gly Tyr Phe Gly Ile Trp Ser

        275         284         293         302         311         320
TTC CCC TTA ATA ATC GCC GCT GTC TGT GCG CAG AGT GTC AAT GAC CCT AGT AAT
Phe Pro Leu Ile Ile Ala Ala Val Cys Ala Gln Ser Val Asn Asp Pro Ser Asn

        329         338         347         356         365         374
ATG TCG CTG GTT AAA GAG ACG GTG GAT AGA CTC CTG AAA GGC TAT GAC ATT CGT
MET Ser Leu Val Lys Glu Thr Val Asp Arg Leu Leu Lys Gly Tyr Asp Ile Arg

        383         392         401         410         419         428
CTG AGA CCA GAT TTT GGA GGT CCC CCC GTG GCT GTG GGG ATG AAC ATT GAC ATT
Leu Arg Pro Asp Phe Gly Gly Pro Pro Val Ala Val Gly MET Asn Ile Asp Ile

        437         446         455         464         473         482
GCC AGC ATC GAT ATG GTT TCT GAA GTC AAT ATG GAT TAT ACC TTG ACA ATG TAC
Ala Ser Ile Asp MET Val Ser Glu Val Asn MET Asp Tyr Thr Leu Thr MET Tyr

        491         500         509         518         527         536
TTT CAA CAA GCC TGG AGA GAT AAG AGG CTG TCC TAT AAT GTA ATA CCT TTA AAC
Phe Gln Gln Ala Trp Arg Asp Lys Arg Leu Ser Tyr Asn Val Ile Pro Leu Asn

        545         554         563         572         581         590
TTG ACT CTG GAC AAC AGA GTG GCA GAC CAG CTC TGG GTG CCT GAT ACC TAT TTC
Leu Thr Leu Asp Asn Arg Val Ala Asp Gln Leu Trp Val Pro Asp Thr Tyr Phe

        599         608         617         626         635         644
CTG AAC GAT AAG AAG TCA TTT GTG CAC GGA GTG ACT GTT AAG AAC CGC ATG ATT
Leu Asn Asp Lys Lys Ser Phe Val His Gly Val Thr Val Lys Asn Arg MET Ile

        653         662         671         680         689         698
CGC CTG CAT CCT GAT GGC ACC GTC CTT TAT GGA CTC AGA ATC ACA ACC ACA GCT
Arg Leu His Pro Asp Gly Thr Val Leu Tyr Gly Leu Arg Ile Thr Thr Thr Ala
```

FIGURE 6 (CONTINUED)

```
        707         716         725         734         743         752
GCC TGC ATG ATG GAC CTA AGG AGG TAC CCA CTG GAT GAA CAA AAC TGC ACC TTG
Ala Cys MET MET Asp Leu Arg Arg Tyr Pro Leu Asp Glu Gln Asn Cys Thr Leu

        761         770         779         788         797         806
GAA ATT GAG AGC TAT GGA TAC ACA ACT GAT GAC ATT GAG TTT TAC TGG CGT GGC
Glu Ile Glu Ser Tyr Gly Tyr Thr Thr Asp Asp Ile Glu Phe Tyr Trp Arg Gly

        815         824         833         842         851         860
GAT GAT AAT GCA GTA ACA GGA GTA ACG AAA ATT GAA CTT CCA CAG TTC TCT ATT
Asp Asp Asn Ala Val Thr Gly Val Thr Lys Ile Glu Leu Pro Gln Phe Ser Ile

        869         878         887         896         905         914
GTA GAT TAC AAA CTT ATC ACC AAG AAG GTT GTT TTT TCC ACA GGT TCC TAT CCC
Val Asp Tyr Lys Leu Ile Thr Lys Lys Val Val Phe Ser Thr Gly Ser Tyr Pro

        923         932         941         950         959         968
AGG TTA TCC CTC AGC TTT AAG CTT AAG AGA AAC ATT GGC TAC TTT ATC CTG CAA
Arg Leu Ser Leu Ser Phe Lys Leu Lys Arg Asn Ile Gly Tyr Phe Ile Leu Gln

        977         986         995        1004        1013        1022
ACA TAC ATG CCT TCC ATC CTG ATT ACC ATC CTC TCC TGG GTC TCC TTC TGG ATT
Thr Tyr MET Pro Ser Ile Leu Ile Thr Ile Leu Ser Trp Val Ser Phe Trp Ile

       1031        1040        1049        1058        1067        1076
AAT TAC GAT GCT TCA GCT GCA AGG GTG GCA TTA GGA ATC ACA ACT GTC CTC ACA
Asn Tyr Asp Ala Ser Ala Ala Arg Val Ala Leu Gly Ile Thr Thr Val Leu Thr

       1085        1094        1103        1112        1121        1130
ATG ACC ACA ATC AAC ACC CAC CTC CGG GAA ACT CTC CCT AAA ATC CCC TAT GTG
MET Thr Thr Ile Asn Thr His Leu Arg Glu Thr Leu Pro Lys Ile Pro Tyr Val

       1139        1148        1157        1166        1175        1184
AAG GCC ATT GAC ATG TAC CTG ATG GGG TGC TTT GTC TTC GTT TTC ATG GCC CTT
Lys Ala Ile Asp MET Tyr Leu MET Gly Cys Phe Val Phe Val Phe MET Ala Leu

       1193        1202        1211        1220        1229        1238
CTG GAA TAT GCC CTA GTC AAC TAC ATC TTC TTT GGG AGG GGG CCC CAA CGC CAA
Leu Glu Tyr Ala Leu Val Asn Tyr Ile Phe Phe Gly Arg Gly Pro Gln Arg Gln

       1247        1256        1265        1274        1283        1292
AAG AAA GCA GCT GAG AAG GCT GCC AGT GCC AAC AAT GAG AAG ATG CGC CTG GAT
Lys Lys Ala Ala Glu Lys Ala Ala Ser Ala Asn Asn Glu Lys MET Arg Leu Asp

       1301        1310        1319        1328        1337        1346
GTC AAC AAG ATG GAC CCC CAT GAG AAC ATC TTA CTG AGC ACT CTC GAG ATA AAA
Val Asn Lys MET Asp Pro His Glu Asn Ile Leu Leu Ser Thr Leu Glu Ile Lys

       1355        1364        1373        1382        1391        1400
AAT GAA ATG GCC ACA TCT GAG GCT GTG ATG GGA CTT GGA GAC CCC AGA AGC ACA
Asn Glu MET Ala Thr Ser Glu Ala Val MET Gly Leu Gly Asp Pro Arg Ser Thr

       1409        1418        1427        1436        1445        1454
ATG CTA GCC TAT GAT GCC TCC AGC ATC CAG TAT CGG AAA GCT GGG TTG CCC AGG
MET Leu Ala Tyr Asp Ala Ser Ser Ile Gln Tyr Arg Lys Ala Gly Leu Pro Arg
```

FIGURE 6 (CONTINUED)

```
         1463          1472          1481          1490          1499          1508
CAT AGT TTT GGC CGA AAT GCT CTG GAA CGA CAT GTG GCG CAA AAG AAA AGT CGC
His Ser Phe Gly Arg Asn Ala Leu Glu Arg His Val Ala Gln Lys Lys Ser Arg

         1517          1526          1535          1544          1553          1562
CTG AGG AGA CGC GCC TCC CAA CTG AAA ATC ACC ATC CCT GAC TTG ACT GAT GTG
Leu Arg Arg Arg Ala Ser Gln Leu Lys Ile Thr Ile Pro Asp Leu Thr Asp Val

         1571          1580          1589          1598          1607          1616
AAT GCC ATA GAT CGG TGG TCC CGC ATA TTC TTC CCA GTG GTT TTT TCC TTC TTC
Asn Ala Ile Asp Arg Trp Ser Arg Ile Phe Phe Pro Val Val Phe Ser Phe Phe

         1625          1634          1643                 1659        1669        1679
                                               >
AAC ATC GTC TAT TGG CTT TAT TAT GTG AAC TAA AACATGGCCT CCCACTGGAA GCAAGGACTA
Asn Ile Val Tyr Trp Leu Tyr Tyr Val Asn  .

       1689       1699       1709       1719       1729       1739       1749
GATTCCTCCT CAAACCAGTT GTACAGCCTG ATGTAGGACT TGGAAAACAC ATCAATCCAG GACAAAAGTG

       1759       1769       1779       1789       1799       1809       1819
ACGCTAAAAT ACCTTAGTTG CTGGCCTATC CTGTGGTCCA TTTCATACCA TTTGGGTTGC TTCTGCTAAG

       1829       1839       1849       1859
TAATGAATAC ACTAAGGTCC TTGTGGTTTT CCAGTTAAAA CGCAAGT
```

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/GB 93/02506

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C07K13/00 C12N5/10 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EUROPEAN JOURNAL OF PHARMACOLOGY vol. 189, no. 1 , 31 July 1990 pages 77 - 88 MOSS SJ;SMART TG;PORTER NM;NAYEEM N;DEVINE J;STEPHENSON FA;MACDONALD RL;BARNARD EA; 'Cloned GABA receptors are maintained in a stable cell line: allosteric and channel properties.' see the whole document ---	1-13
X	US,A,5 166 066 (CARTER, D.B.) 24 November 1992 see the whole document ---	1-9,12, 13
P,X	WO,A,92 22652 (MERCK SHARP & DOHME LTD.; GB) 23 December 1992 see the whole document -----	1-13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
17 March 1994	08. 04. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nauche, S

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 93/02506

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5166066	24-11-92	NONE		
WO-A-9222652	23-12-92	AU-A-	1921192	12-01-93
		CA-A-	2109193	12-12-92